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THE ARBOVIRUSES

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THE ARBOVIRUSES

[Following is a translation of a portion of a book Virus- und Rickettsieninfektionen des Menschen (Viral and Rickettsial Infections of Man) by H. Moritsch, Munich, pp 412-494.]

Abbreviations

Ag	= Antigen	KFD	= Kyasanur Forest Disease
AHS	= African Horse Sickness	LI	= Louping Ill
Ab	= Antibodies	ME	= Meningoencephalitis
Arbo	= Arthropod-borne	MVE	= Murray Valley Encephalitis
BTS	= Blue Tongue of Sheep	NDV	= Newcastle Disease Virus
CE	= California Encephalitis	NA	= Nucleic Acid
CEE	= Central European Enceph-alitis	NT	= Neutralization Test
		OHF	= Omsk Hemorrhagic Fever
CPE	= Cytopathic effect	RES	= Reticuloendothelial System
CTF	= Colorado Tick Fever	RNA	= Ribonucleic Acid
EEE	= Eastern Equine Encephalitis	RSSE	= Russian Spring-Summer Encephalitis
EHD	= Epidemic Hemorrhagic Disease	RVF	= Rift Valley Fever
ESME	= Early-Summer Meningo-Encephalitis	s.c.	= subcutaneous
		SLE	= St. Louis Encephalitis
HA	= Hemagglutinin	TBE	= Tick-Borne Encephalitis
HF	= Hemorrhagic Fever	VEE	= Venezuelan Equine Encephalitis
HIT	= Hemagglutination-Inhibition Test	VSV	= Vesicular Stomatitis Virus
IF	= Interferon	WEE	= Western Equine Encephalitis
i.c.	= intracerebral	WN	= West Nile
JBE	= Japanese B Encephalitis	YF	= Yellow Fever
CFS	= Complement-Fixation Reaction		

GENERAL

A. Definition

Viruses are now classified as arthropod-borne (arbo) viruses which:

(1) Possess the capacity to multiply in vertebrates and arthropods, with the vertebrates regarded as the reservoir and the arthropods as the vector. Multiplication in vertebrates is traceable through viremia and accompanying antibody formation. The infection may either be inapparent or have clinically pronounced symptoms. The virus itself can be eliminated by lactating animals through milk; in excrement it has thus far been found only in mice infected with the VEE virus. Infection of a vertebrate follows the bite of an arthropod which had previously become infected during a blood meal on a viremic vertebrate. Experimental mosquitoes can be infected intrathoracically, with the demonstrable multiplication of the virus considered a criterion for classification with the mosquito-borne arboviruses. Virus multiplication in arthropods is always asymptomatic, i.e., there are no signs of disease or histologically apparent lesions. Consequently, the so-called "insect viruses" (= viruses which multiply in insects and thereby injure them) and viruses transmitted only mechanically by arthropods are <u>not</u> designated arboviruses.

(2) Multiply in baby mouse brain.

(3) Are sensitive to bile salts ~~[190]~~.

(4) Possess hemagglutin of antigenic nature.

(5) Contain RNA.

All the postulates (especially the production of HA) for classification among the arboviruses cannot always be satisfied. In the case of the Entebbe bat virus isolated in Uganda from the salivary glads of a bat (<u>Tadarida</u> [Chaerephon] <u>limbata</u> Peters), we know only that it can be concentrated in the baby mouse and the resultant HA (cf. section on Immunobiology) gives a cross reaction with immune sera of group B without the possibility of identification with suitable known sera. There is no hint of a vertebrate-arthropod cycle in nature. Nevertheless, such viruses are included with the arboviruses on the basis of the antigenic relationship (at least tentative).

A definition of arboviruses from the physical standpoint, however, is impossible because they are heterogeneous in size and presumably also possess a variable number of capsomeres.

Most arboviruses can be divided into separate groups on the basis of antigen relationship. The remaining arboviruses are tentatively treated as "ungrouped". Those viruses are related and placed in one group [32] which produce clear cross reactions either in the HIT or CFR. A precise differentiation of the individual strains is then possible in the HIT and CFR only through the essentially higher titer with the homologous sera, otherwise - apart from the TBE complex - in the NT, where, to be sure, cross reactions can also occur. These, however, prove to be very weak (e.g., with concentrated or only slightly attenuated serum) and can thus be easily differentiated by neutralization with the homologous serum.

In general, only the HIT is used for grouping, as, for example, for the division into group A or B; for the grouping in C, however, a combination of the HIT and CFR (diagram) [167].

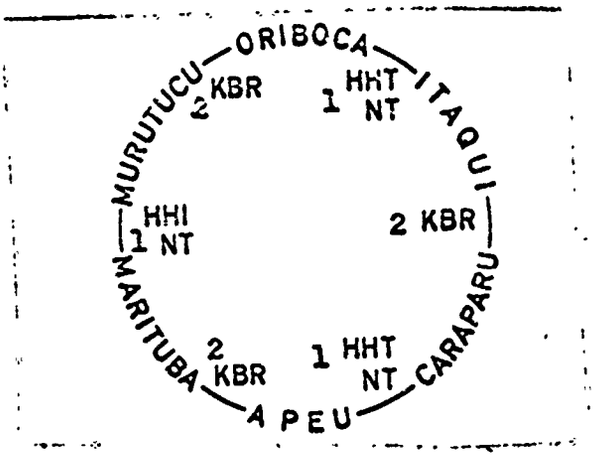


Fig. 1. Serological relations of the group C viruses. Diagram from R. E. Shope and O. R. Causey, Amer. J. Trop. Med. Hyg., Vol 11, pp 283-290, 1962.

1 - Hemagglutination-inhibition test
2 - Complement-fixation reaction

Beyond that, however, viruses closely related in antigen structure within a group can also be included in a complex, as in the TBE complex within the B group. Here a precise differentiation of the individual types is possible only through such delicate methods as cross precipitation in agar gel and the HIT with type-specific (obtained through cross absorption) hyperimmune sera [40] or by the combined use of immunofluorescence and microphotometry [68a].

This grouping in the case of many of the already discovered arboviruses and familiar groups is a complicated and protracted procedure. Another difficulty that occasionally arises is that there are some individual virus strains which produce cross reactions

with two groups and appear to be a bridge between them:

virus group		virus strain		virus group
	cross reaction		cross reaction	
Bunyamwera	←——— CFR ———→	GUAROA	←——— HIT ———→	California encephalitis

In spite of all these difficulties, the serological classification method offers the only available possibility of arranging these viruses according to constant and stable characteristics.

The wish, expressed perhaps mostly by clinicians, to be able to classify viruses by the clinical symptoms appearing in man or animals is impossible in the case of the arboviruses because (1) these criteria are not constant and (2) the clinical picture cannot be as exactly perceived as the antigen structure of a virus so that misinterpretations are possible which are reliably excluded by antigen analysis.

B. Classification

The tables list the presently known arboviruses in groups, which were originally designated by A, B, and C. This method was abandoned with the discovery of the fourth group (1960) and each group is designated by the name of the first disdovered virus. The viruses mentioned in detail correspond to a single type. Within each type subtypes (variants) can be distinguished [31a] on the basis of a refined HIT.

C. Physical, Chemical and Biological Characteristics

1. Physical Characteristics

Most arboviruses are 20-50 mμ in size, as measured by the ultracentrifuge and electron microscope. Some exceptions with a larger diameter are the blue tongue, Ilesha, Turlock, Wyeomia, Anopheles A and B, Bunyamwera, Bwamba, Ntaya, and VEE viruses. This figure applies to the overall size of the elementary particle. It is independent of the diameter of the external membrane, which has a larger range of variation than the dimensions of the structural elements of the inner body.

2. Chemical Characteristics

According to R. M. Franklin [52], arboviruses are viruses with peripherally situated structural lipids. They lose their infectiousness very quickly after treatment with chloroform [110, 209], ethyl ether (18 hours at 4° C), or Na-desoxycholate (0.1% for 30 min at 22° C). Inactivation is also regarded as an essential characteristic of the arboviruses.

Also, S. G. Anderson and G. L. Ada's investigations [3], wherein phospholipase A inactivated the MVE virus, indicate that peripherally situated phospholipids must be essential for the integrity of the virus. The lipid content of this virus was later determined accurate to 11% [1a]. Infectious RNA can be obtained if these lipids are removed together with the proteins by treatment with hot carbolic acid (50° C) [202]. Treatment with Freon, among others, does not alter the infection titer proper so that this method can be used to purify these viruses too [177]. The production of infectious ribonucleic acid with the help of extraction with cold carbolic acid mentioned by Gierer and Schramm has already been successfully attempted in West Nile [43], Murray Valley [1], TBE [47, 178], WEE and EEE [108, 200, 201, 203], Semliki Forest [37], and yellow fever [125].

Key to Following Tables

1 - geographical occurrence; 1a - ( ) conjectural; 2 - isolation from; 3 - Man; 4 - human antibodies; 5 - form of disease; 6 - unknown; 7 - man: dengue-like, often with HF; 8 - man: hem. ME; 9 - ** species unknown; 10 - *The internationally used English names have been chosen to designate the viruses; 11 - man: dengue-like; 12 - man: fever; 13 - man: fever (rarely with ME?); 14 - man: ME; 15 - man: fever (with hepatitis?); 16 - sheep: fever; 17 - man: HF; 18 - man: fever (laboratory infection); 19 - man: dengue; 20 - man: dengue, also HF; 21 - (related to MVE virus); 22 - man: fever (with ME?); 23 - turkey: ME; 24 - horses: ME; 25 - (do not multiply in mosquitoes); 26 - man: fever (dengue-like?); 27 - sheep: fever with abortion; 28 - man: dengue-like (with myocarditis) ME; 29 - man: yellow fever; 30 - (laboratory infection); 31 - man: fever (related to the California encephalitis complex); 32 - swine: African swine fever?); 33 - sheep: catarrh, fever, edema, hem. diathesis; 34- man?; 35 - man: pappataci fever; 36 - influenza-like; 37 - domestic animals: vesicular stomatitis; 38 - man: febrile infection (?); 39 - sheep: gastroenteritis with glomerulonephritis; 40 - man: fever (laboratory infection); 41 - sheep: hepatitis with abortion; 42 - man: fever (dengue-like?); 43 - man: dengue-like; 44 - sheep: louping ill; 45 - South Africa; 46 - East Africa; 47 - South America; 48 - East Asia; 49 - Eastern United States; 50 - Northern Europe; 51 - South Central Sweden; 52 - Southern Norway; 53 - Central Europe: East Germany (Southern Germany), East-Southeast-Austria, Poland, Slovenia, Hungary, Czechoslovakia; 54 - Southeastern Europe: (Albania, Bulgaria, Greece, Rumania); 55 - Eastern Europe: Europe, Russia; 56 - Southeast Asia; 57 - genusspecies (genus and species unknown); 58 - * vertebrates freely exposed; 59 - Anterior Asia; 60 - Greece; 61 - Cyprus; 62 - Czechoslovakia, Austria, Jugoslavia, (Southeast Europe?); 63 - Near East; 64 - Sicily; 65 - Czechoslovakia; 66 - Far East

Gruppe A

Virus*	1a geogr. Vorkommen () vermutet	2 *Isolierung* aus Mensch	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
		3					
Aura	Äquatorial-Amerika			+		Culex sp.** Aedes sp.	
Bebaru	Malaya			+	+	Culicinae div.gen.***	
Chikungunya		+		+	+	Culicinae div.gen.	7 Mensch: Dengue ähnlich, manchmal mit HF
Subtypen:							
Afrika	Tanganyika, Kongo, Süd-Afrika, Ost-Afrika						
Thailand	Thailand 43 46						
Eastern Equine Encephalomyelitis (EEE)		+	+	+	+	Culiseta melanura, Culicinae div. gen., Culicoides sp.	8 Mensch: haem. ME Equide: ME.
Subtypen:							
Nord-Amerika	49 Kanada, Ost-USA, Jamaika, Dominikanische Republik						
Zentral-Süd-Amerika	Panama, Britisch Guyana, Trinidad, Brasilien, Argentinien						
Getah	48 Ost-Asien, Australien		—	+	+	Culicinae div. gen.	
Highlands J.	Florida		+		6	unbekannt	

10 * Für die Bezeichnung der Viren wurden die internatonal verwendeten englischen Namen gewählt. 9 ** Species unbekannt. *** diverse genera.

Mayaro	Äquatorial-Amerika, (Süd-Amerika)	+		+	+	Mansonia venezuelensis	15 Mensch: Fieber (mit Hepatitis?)
Middelburg	47 45 Süd-Afrika			+	+	Aedes div sp.	16 Schafe: Fieber (?)
Ndumu	45 Süd-Afrika			+	+	Aedes sp. Mansonia sp.	
O'nyong-nyong	46 Ost-Afrika (Kongo, Sudan)	+		+	+	Anopheles div. sp.	11 Mensch: Dengue-ähnlich
Pixuna	Äquatorial-Amerika		+	+		Anopheles nimbus	
Semliki Forest	46 West-, Ost- u. Äquatorial-Afrika			+	+	Aedes div. sp.* Eratmapodites sp.	
Sindbis		+	+	+	+		
Subtypen: Australien	Australien 45					Culicinae div. gen.	12 Mensch: Fieber
Afrika	Ägypten, Süd-Afrika						
Ferner Osten	Malaya, Indien, Philippinen						
Una	Äquatorial-Amerika			+	+	Culicinae div. gen.	
Venezuelan Equine Encephalomyelitis (VEE)	Äquatorial-Amerika	+	+	+	+	Culicinae div. gen.	13 Mensch: Fieber (in seltenen Fällen mit ME?) Equide: ME
Western Equine Encephalomyelitis (WEE)	Kanada, Westl. USA, Äquatorial-Amerika, Argentinien	+	+	+	+	Culex tarsalis (Culicinae div. gen.)	14 Mensch: ME Equide: ME

Gruppe B (TBE-Komplex)

Virus	1 geogr. Vorkommen 1a ( ) vermutet	2 *Isolierung* aus Mensch	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Tick borne Encephalitis (TBE)							
Subtyp: Central European Encephalitis (CEE) = Früh-sommer-Meningo-Enzephalitis (FSME)	50 *Nord-Europa:* Ålands-Archipel, (Bornholm), Süd-Mittel-Schweden, 51 (Süd-Norwegen) 52 53 *Mittel Europa:* Ost-Deutschland, (Süd-Deutschland), Ost-Südost-Österreich, Polen, Slowenien, Ungarn, ČSR	+	+	+	+	Ixodes ricinus	14 Mensch: ME
	54 *Südost-Europa:* (Albanien, Bulgarien, Griechenland, Rumänien)						
	55 *Ost-Europa:* Europ. Rußland						

Subtyp: Russian Spring Summer Encephalitis (RSSE)	Ferner Osten, Sibirien	+	+	+	+	Ixodes persulcatus (Ixodes ricinus, Dermacentor silvarum, Haemaphysalis concinna)		ch: ME
Kyasanur Forest Disease (KFD)	Indien	+	+	+	+	Haemaphysalis spinigera (Haemaphysalis div. sp.)		ch: HF
Langat	Malaya			+	+	Ixodes granulatus		
Louping ill	Nord-England, Irland, Schottland, Wales	+	+	+	+	Ixodes ricinus		ch: LI ch: ME
Negishi	Japan	+				unbekannt		Mensch: ME
Omsk-Hem. Fever Subtyp I Subtyp II	Sibirien Zentral-UdSSR	+		+	+	Dermacentor pictus Dermacentor marginatus		Mensch: HF
Powassan	Kanada, USA	+	+	+	+	Ixodes marxi Dermacentor andersoni		Mensch: ME

Gruppe B

Virus	1 geogr. Vorkommen (1a) () vermutet	2 *Isolierung* aus Mensch (3)	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Bat Salivary Gland Virus = Rio Bravo	Mexiko, USA		+			6 unbekannt	18 Mensch: Fieber (Laborinfektion)
Bussuquara	Äquatorial-Amerika		+	+		Culex sp.	
Dengue I	Hawaii, Indien, Malaya, Neu-Guinea, (Süd-Afrika, Australien, Ost-Asien, Griechenland) 45 48	+			+	(Aedes aegypti)	19 Mensch: Dengue
Dengue II	Neu-Guinea, Thailand, Trinidad, Indien, (Äquatorial-Amerika, Indonesien, Malaya)	+		+	+	Aedes aegypti (Culicinae div. gen.)	20 Mensch: Dengue, auch HF
Dengue III	Philippinen, Thailand	+		+	+	Aedes aegypti (Culicinae div. gen.)	17 Mensch: HF
Dengue IV	Philippinen, Thailand, Indien	+			+	6 unbekannt	17 Mensch: HF
Edge Hill	Australien			+		Culicinae div. gen.	21 (verwandt mit MVE-Virus)
Entebbe Bat	Uganda		+			6 unbekannt	

Gelbfieber (siehe Yellow Fever)							
H-336 45	Süd-Afrika	+		+	+	Culex rubinotus 12	Mensch: Fieber
Ilheus	Äquatorial-Amerika, Mittel-Amerika, (Südost-Asien) 56	+	+	+	+	Culicinae div. gen 22	Mensch: Fieber (mit ME)
Israel Turkey Meningo Enc.	Israel		+			6 unbekannt 23	Truthahn: ME
Japan B. Enc. (JBE)	Japan, 56 48 (Südost- u. Ost-Asien, Indien, Mikronesien)	+	+	+	+	Culex tritaeniorhynchus 14 24 (Culicinae div. gen.)	Mensch: ME Pferde: ME
Kokobera	Australien			+		Culex annulirostris	
Kunjin	Australien			+	+	Culex annulirostris	
MML	Montana		+			6 unbekannt	
Modoc	Kalifornien		+			6 unbekannt 25	(in Moskitos nicht vermehrbar)
Murray Valley Encephalitis 56 (MVE)	Australien, Neu-Guinea, (Südost-Asien)	+		+	+	Culex annulirostris 14 (Culicinae div. gen.)	Mensch: ME
Ntaya	Uganda, (Südost-Asien) 56			+	+	Culicidae gen. sp.*	

57* genus species (Genus und Spezies unbekannt).

Virus	geogr. Vorkommen (1) (1a) () vermutet	Isolierung aus (2) Mensch (3)	Vertebrat	Arthropode	Antikörper Mensch (4)	Vektor	Krankheitsbild (5)
St. Louis Encephalitis (SLE)	USA, Äquatorial-Amerika, (Südost-Asien)	+	+	+	+	Culex div. sp. (Culicinae div. gen.) (14)	Mensch: MF.
Spondweni	Nigeria, Süd-Afrika, (Ost-Afrika) (44) (45)	+		+		Culicinae div. gen. (12)	Mensch: Fieber
Stratford	Australien (46)			+		Aedes vigilax	
Tembusu	Malaya			+		Culicinae div. gen.	
Uganda S	Süd- u. Ost-Afrika, (West-Afrika, Indien, Indonesien) (45) (46)	+		+	+	Culicinae div. gen. (26)	Mensch: Fieber (Dengue-ähnlich?)
Usutu	Süd-Afrika (45)			+		Culex univittatus	
Wesselsbron	Süd- u. Ost-Afrika (45) (46)	+	+	+	+	Culicinae div. gen. (26) (27)	Mensch: Fieber (Dengue-ähnlich) Schafe: Fieber mit Abort
West Nile (WN)	Ägypten, Uganda, Borneo, Indien, Israel, (Philippinen, Kongo, Sudan)	+	+	+	+	Culex div. sp. (Culicinae div. gen.) (28)	Mensch: Dengue-ähnlich (mit Myokarditis) ME

12

Yellow Fever (YF)		+	+	+	+		29	Mensch: Gelbfieber
Subtypen:								
America	Trinidad, Brasilien, Mittel-Amerika					Haemagogus div. sp. (Culicinae div. gen.)		
Africa	West-Afrika, Äquatorial-Afrika					Aedes aegypti (Aedes div. sp.)		
Zika	45 Ost-Afrika, (Nigeria, Indien, Indonesien, Malaya, Philippinen)	+	+	+	+	Aedes africanus	12	Mensch: Fieber
			Gruppe C					
Apeu	Äquatorial-Amerika	+	(+)*		+	6 unbekannt	12	Mensch: Fieber
Caraparu	Brasilien, West-Indien	+	(+)	+	+	Culicinae gen. sp.		
Itaqui	Äquatorial-Amerika	+	+		+	6 unbekannt	12	Mensch: Fieber
Marituba	Äquatorial-Amerika	+	(+)		+	6 unbekannt	12	Mensch: Fieber
Murutucu	Äquatorial-Amerika	+	+		+	6 unbekannt	12	Mensch: Fieber
Nepuyo	Äquatorial-Amerika			+	+	Culex sp.		
Oriboca	Äquatorial-Amerika, (Süd-Afrika, Angola) 45	+	+	+	+	Culicinae div. gen.	12	Mensch: Fieber

58 * = im Freiland exponierte Vertebraten (+) = (sentinel animals)

Gruppe Bunyamwera

Virus	1 geogr. Vorkommen 1a () vermutet	2 Isolierung aus Mensch 3	2 Isolierung aus Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Batai = Chittoor = Calovo	Indien, Malaya, CSR, (Österreich)			+	+	Culex gelidus (Batai), Anopheles barbirostris (Chittoor), Anopheles maculipennis (Calovo)	
Bunyamwera	46 45 Ost- u. Süd-Afrika, Nigeria, (Kongo)	+		+	+	Aedes div. sp.	12 Mensch: Fieber
Cache Valley	Äquatorial-Amerika, USA			+	+	Culicidae div. gen.	
Germiston 45	Süd-Afrika, (Angola)			+	+	Culex div. sp.	30 (Laborinfektion)
Guaroa	Äquatorial-Amerika, (Süd-Amerika)	+		+	+	Anophelinae gen. sp	31 Mensch: Fieber (verwandt mit California Encephalitis-Komplex)
Ilesha	Nigeria	+			+	6 unbekannt	12 Mensch: Fieber
Kairi	Äquatorial-Amerika			+	+	Culicinae div. gen.	
Sororoca	Äquatorial-Amerika			+		Culicinae gen. sp.	
Wyeomyia	Äquatorial-Amerika			+		Culicinae div. gen.	

Gruppe Anopheles A						
Anopheles A	Amerika		+		Anopheles div. sp.	
Lukuni	Äquatorial-Amerika		+	+	Culicinae div. gen.	
Gruppe African Horse Sickness						
African Horse Sickness (AHS) 59	Afrika, Indien, Vorder-Asien, Griechenland, Zypern 60 61		+	+	Culicoides sp.	Equiden: AHS Schweine: African swine fever ? 32
Gruppe Bakau						
Bakau	Malaya		+		Culex lophoceratomyia	
Ketapah	Malaya		+		Culex lophoceratomyia	
Gruppe Blue Tongue						
Blue Tongue of Sheep (BTS)	Afrika, Japan, Pakistan, Portugal, Spanien, USA, Zypern 61		+	+	Culicoides sp.	Schafe: Katarrh, Fieber, Ödeme, Häm. Diathese 33

Gruppe Bwamba

Virus	1 geogr. Vorkommen 1a ( ) vermutet	2 *Isolierung aus* Mensch 3	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Bwamba	Uganda, (Afrika)	+			+	6 unbekannt	12 Mensch: Fieber
Pongola	Süd- und Ost-Afrika 45 46			+	+	Culicinae div. gen.	

Gruppe Californi: Encephalitis

California Encephalitis (CE)	West-USA		+	+	+	Culex tarsalis Culicinae div. gen.	14 Mensch: ME?
Lumbo	46 Ost-Afrika			+	+	Aedes pembaensis	
Melao	Äquatorial-Amerika			+		Aedes scapularis	
Tahyna	62 CSR, Österreich, Jugoslawien, (Süd-Ost-Europa?)	+		+	+	Aedes vexans Aedes caspius	34 Mensch ?
Trivittatus	USA			+	+	Aedes trivittatus	

				Gruppe Capim			
Bushbush	Äquatorial-Amerika			+			Culicinae gen. sp.
Capim	Äquatorial-Amerika		+			6	unbekannt
Guajara	Äquatorial-Amerika		(+)			6	unbekannt
				Gruppe Guama			
Be An 20525	Äquatorial-Amerika		(+)			6	unbekannt
Bimiti	Trinidad			+	+		Culex sp.
Caru	Äquatorial-Amerika	+	+	+			Culex mojuensis 12 Mensch: Fieber
Guama	Äquatorial-Amerika	+	+	+	+		Culicinae div. gen. 12 Mensch: Fieber
Moju	Äquatorial-Amerika			+			Culex sp.
				Gruppe Irituia			
B T 436	Panama			+			Phlebotomus sp.
Irituia	Äquatorial-Amerika		+	+			Phlebotomus sp.

Virus	1 geogr. Vorkommen 1a ( ) vermutet	2 Isolierung aus Mensch 3	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Gruppe Koongol							
Koongol	Australien			+		Culicinae div. gen.	
Wongal	Australien			+		Culex annulirostris	
Gruppe Naples Phlebotomus Fever							
Icoarari	Brasilien		+			6 unbekannt	
Sandfly Naples	Italien, Naher Osten 63	+				(Phlebotomus papatasi)	35 Mensch: Pappataci-Fieber
Sandfly Sicilian	64 Sizilien	+		+	+	Phlebotomus papatasi	35 Mensch: Pappataci-Fieber
Gruppe Simbu							
Akabane	Japan			+		Culicinae div. gen.	
Ingwavuma	45 Süd-Afrika		+	+		Culex univittatus	
Manzanilla	Trinidad		+			6 unbekannt	
Oropouche	Äquatorial-Amerika	+		+	+	Culicinae div. gen.	12 Mensch: Fieber
Sathuperi	Indien			+		Culex vishnui	
Simbu	45 Süd-Afrika			+		Aedes circumluteolus	

				Gruppe Tacaribe				
Junin	Argentinien	+	+	+	+	Echinolaelaps echidninus (?)	17	Mensch: HF
Tacaribe	Äquatorial-Amerika		+	+		Culicinae gen. sp.		
				Gruppe Triniti				
Aruac	Trinidad			+		Tridioprosopon theobaldi		
Jeri	Trinidad			+		Psorophora albipes		
Triniti	Trinidad			+	+	Tridioprosopon sp.		
				Gruppe Turlock				
Turlock	Kalifornien		+	+		Culex div. sp.		
Umbre	Indien			+		Culex div. sp.		
				Gruppe Vesicular Stomatitis				
Coeal	Äquatorial-Amerika		+	+		Gigantolaelaps sp. (?)		
VS-Indiana	Indiana, USA	+	+	+	+	Phlebotomus sp. (?)	36	Mensch: Influenza-ähnlich
VS-New Jersey	New Jersey		+				37	Haustiere: Vesikulire Stomatitis

Ungruppiert

Virus	1 geogr. Vorkommen 1a () vermutet	2 *Isolierung aus* Mensch 3	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Acara	Äquatorial-Amerika		(+)			6 unbekannt	
Anopheles B	Äquatorial-Amerika			+		Anopheles boliviensis	
Candiru	Äquatorial-Amerika	+				6 unbekannt	11 Mensch: Dengue-ähnlich
Chenuda	Ägypten, Süd-Afrika			+		Argas div. sp.	
Colorado Tick Fever (CTF)	West-USA	+	+	+	+	Dermacentor andersoni, Ixodidae div. gen.	12 Mensch: Fieber
EG AR 492	Sudan, Indien			+		Rhipicephalus sanguines, Hyaloma aegypticum	
EG AR 1304	45 Ägypten, Süd-Afrika		+	+		Argas persicus	
Epizootic Hemorrhagic Disease of Deer (EHD)	USA		+			6 unbekannt	

Hart Park	Kalifornien		+	+		Culex tarsalis		
Hughes	Florida, Trinidad			+		Ornithodorus sp.		
Kemerovo	West-Sibirien, CSR, Finnland 65	+	+	+	+	Ixodes persulcatus, Ixodes ricinus 38	Mensch: Fieberhafter Infekt (?)	
Kern Canyon	Kalifornien		+			6 unbekannt		
Lagos Bat	Nigeria		+			6 unbekannt		
Mapputta	Australien			+		Anopheles meraukensis		
Mirim	Äquatorial-Amerika		(+)	+		Culex sp.		
Mossuril 46	Ost-Afrika			+	+	Culex div. sp.		
MP 401	Kongo			+		Anopheles funestus		
Nairobi Sheep Disease	Äquatorial-Afrika, (Süd- u. Ost-Afrika) 45 46		+	)	+	Rhipicephalus appendiculatus 39	Schafe: Gastro-Enteritis mit Glomerulonephritis	
Nakiwogo 46 45	Ost-Afrika, (Süd-Afrika)	+				6 unbekannt	14 Mensch: ME	
Pacui	Äquatorial-Amerika		+			6 unbekannt		

Virus	1 geogr. Vorkommen 1a( ) vermutet	2 Isolierung aus Mensch 3	Vertebrat	Arthropode	4 Antikörper Mensch	Vektor	5 Krankheitsbild
Piry	Brasilien	(+)*	+		6	unbekannt	40 * Mensch: Fieber (Laborinfektion)
Quaranfil	Ägypten, Süd-Afrika 45	+	+	+	+	Argas persicus	
Rift Valley Fever (RVF)	Süd- und Ost-Afrika, (Afrika) 45 46	+	+	+	+	Culicinae div. gen.	41 Schafe: Hepatitis mit Abort 42 Mensch: Fieber (Dengue-ähnlich ?)
Semunya	Uganda	+			6	unbekannt	43 Mensch: Dengue-artig
Silverwater	Kanada			+		Haemaphysalis leporis palustris	
Sogoto	Kenya			+		Ixodidae gen. sp.	
Tacaiuma	Äquatorial-Amerika		(+)	+	+	Haemagogus sp.	
Witwatersand	Süd-Afrika, Ost-Afrika) 45 46			+	+	Culex rubinotus	

3. Biological Characteristics

(a) Antigen Structure

Basically, a distinction must be made between three antigens identifiable by various serological methods.

(1) The V(-irus) antigen corresponds to the complete infectious virus particle and is quantitatively determined by the NT.

(2) Up to now hemagglutinin (HA) could not be separated from the virus particle. Hallauer (1946) was the first to obtain proof of HA in a viscerotropic yellow-fever strain. Sabin et al. (1950-1954) subsequently demonstrated the presence of this HA in some arbovirus strains and Casals and Brown [32] used HA to classify the arbovirus strains into groups. Today it is obtained by sucrose-acetone or acetone-ether (standard regulation) extraction from the brain of infected baby mice [39]. This extraction takes place in the cold so that the infectiousness of the virus particle is not completely lost. Hemagglutination is very pH-sensitive and can also be inhibited by phospholipids, as they are found, for example, in every serum [138, 157, 159, 160].

The highest and most reliable titer values of hemagglutination are obtained with goose or rooster erythrocytes [152]. Agglutinability of the erythrocytes appears to be dependent on the hormone content of the donor [155, 156]. According to Salminen, who carefully studied the kinetics in TBE viruses, in the case of adsorption and elution by arboviruses it is not a matter of an enzymatic process as in the myxoviruses, but the high rate of elution with high pH values points to electrostatic influences [155]. Studies on inhibitors of hemagglutination in the TBE virus [159, 160] and later in other arboviruses [157] made it likely that as far as the receptors of the cell surface are concerned, it is a question of a complex of chloresterol and a negatively loaded lipid (free fatty acid or phosphatide).

(3) The complement-fixing antigen is quantitatively determined in the CFR, wherein, however, the titer is approximately only one-tenth that in the HIT. Even simple preparations (veronal buffer suspension from the brains of intracerebrally infected suckling mice) produce specific deviations in the CFR and are of practical value, above all, for rapid identification of freshly isolated virus strains. Still high antigen titers are obtained after extraction of the inhibiting lipids, e.g., with sucrose-acetone in the cold, as in the production of HA [39].

(b) Host Spectrum

It is a common characteristic of all arboviruses that they can be concentrated in the baby white mouse. After extraneural application many strains multiply in the CNS in the form of an encephalitis. Adult mice, especially the "neurotropic" strains are, in general, also

sensitive, but much less so. Hence, if possible, only baby mice should be used for the isolation of viruses, especially those of unknown strains, from arthropods. All other vertebrates react to natural or artificial infection by forming antibodies so that such an infection can proceed in the guise of familiar diseases.

Instead of animals, increasing use is made today of tissue culturefor virus concentration. Most arbovirus strains multiply readily in tissue cultures after adaptation. Among others, fresh chick embryo, hamster kidney, and HeLa cells have proven to be very satisfactory for this purpose. A CPE did not, of course, always appear. Therefore, tissue culture is, in general, less used for isolation, but it is finding increasing application in serological diagnosis.

(c) Variations

The appearance of mutants which differ in genotype and phenotype from the original viruses is also possible in arboviruses, in which case various circumstances, e.g., alteration of the virus cycle in nature, necessarily increases the mutation rate. Inclusion in a new reservoir, and presumably even more the transmission to another arthropod, should be of considerable significance and cause the virus to adapt.

Insofar as these stable changes can be serologically objectified, they are expressed in the form of subtypes. However, fine differences between the individual types of the TBE complex might be mentioned here. What is surprising is that in individual types sometimes other arthropods appear as vectors (cf. TBE complex).

A mutation in the direction of a weakening can also be obtained, thus opening up the possibility of producing attenuated strains for vaccination purposes. We might mention the attenuated 17 D (ASIBI) and Dakar strains, which were weakened through passages in chick embryo tissue cultures and in mice and are now used as genetically stable yellow-fever vaccines for man. On the other hand, the Langat TP 21 strain of the TBE complex (q.v.), isolated from ticks, proved to be non-neurotropic for man and is therefore a possible vaccinal strain [59, 141].

(d) Interference

The ability of arboviruses to produce interferon (IF) (cf. the chapter "Interference-Interferon" on p. 202 of the book from which the translated portion was taken) has been demonstrated for several strains. Vilcek [195] reports that he succeeded in concentrating a TBE virus strain without a CPE in tissue cultures from chick embryo fibroblasts and thus demonstrably suppressed the multiplication of the WEE virus (=challenge virus) added 48 hours later. Together with

Zemla and Rada he subsequently showed on the basis of numerous physicochemical studies that interferon was involved [196, 197, 198, 199, 212]. Other IF-forming arboviruses are Chikungunya, O'nyong-nyong, Kumba [147a], Sindbis [72a], EEE [190a], WEE [98a], and vesicular stomatitis [42b, 199b] viruses.

Apparently the homologous and heterologous interference phenomena described by various authors are also to be related to the formation of IF. Lennette and Koprowski [91a] observed back in 1946 that the yellow-fever strain 17 D could suppress in a tissue culture not only the Asibi yellow-fever strain, but also other arboviruses, West Nile. and VEE. The formation of influenza A was suppressed by West Nile, but influenza A did not appear to be able to suppress the formation of the above arboviruses. WEE virus exhibits both homologous interference [98b] and heterologous interference with NDV [91b]. Also various strains of vesicular stomatitis virus interfere with one another [42a]. TBE virus inhibits poliovirus [2a], while Mayaro virus is effective against Sindbis virus [70].

Arboviruses are also sensitive to interferon. Taylor [188a] stated that influenza A interferes with EEE and WEE viruses. Another myxovirus, NDV, suppressed both VEE virus [85a] and WEE virus [91a]. Rabies virus suppressed WEE virus. Vesicular stomatitis virus was suppressed by polyoma virus [46a] and, as already mentioned [70], Sindbis virus is sensitive to IF from Mayaro virus.

It is noteworthy that only inactivated Mayaro virus and Mayaro virus inactivated by deoxycholate have this interfering effect. Inactivation by heat, ultraviolet rays, or antiserum destroys the capacity for interference. The TBE virus' capacity to form IF is likewise destroyed by heat inactivation.

Mayer et al. [107] showed that in chick embryo cells treated beforehand with IF the latent period of the EEE virus was clearly lengthened. Since this occurred not only after inoculation with intact virus but also with infectious RNS from this virus, it is reasonable to assume that IF influences virus synthesis only after removal of the protein coat.

D. Pathogenesis and Clinical Aspects

The mode of human infection under natural conditions is through the bite of a blood-sucking arthropod where the virus is eliminated with the saliva of the arthropod. Other routes of infection are occasionally observed. The percutaneously and passively introduced virus should, by analogy with the dissemination of the virus in experimental animals, be transported through the lymph fluid to the "organ of primary affinity" [109]. This appears to be, at least for TBE, to be the regional lymph nodes in which the virus initially multiplies, thus triggering the first phase of the frequently biphasic

course of the arbovirus infection during which the virus is released into the blood vessels as a result of the primary multiplication. Thus, in experiments on sensitive animals like mice [103, 106] and sheep [104, 102], in contrast to rabbits [105], one can determine the scale on which the virus starts to multiply in the regional lymph nodes after a few hours, before the TBE virus is released into the blood vessels. Furthermore, if the virus is taken per os, it reaches the spleen first after a resorptive viremia where it is concentrated and later released into the blood stream [120].

It is not known whether the development observed in animal experiments can in all cases be applied to man. It is no argument against it that this must be assumed at least for those viruses in which the first phase is clinically inapparent or is accompanied only by mild symptoms like fever (up to 38° C) together with the characteristic general malaise. Were this first phase to proceed with specific clinical symptoms, e.g., exanthema (dengue, Chikungunya, O'nyong-nyong, West Nile [204]), or a hemorrhagic fever, tissue damage would have to be assumed as the cause of the hemorrhagic diathesis in the latter case. At autopsy one finds, to be sure, the changes characteristic of all HF, e.g., hyperemia in enlarged vascular regions, edema, and hemorrhages in all tissues as well as perivascular infiltrates of large mononuclear cells and a striking proliferation of RES cells. It is still unclear, however, whether the virus here has directly attacked the endothelial cells or it involves a hemodynamic reaction to be explained biochemically as in a case of shock [132].

The first phase, independently of the clinical course and quite apart from all succeeding complications, represents the actual "main disease" of the arbovirus infection. In most cases the infection heals without complications, and often only the presence of neutralizing antibodies is the sole indication of infection by an arbovirus. However, it may happen that the virus which circulates in the blood during phase 1 penetrates into the cells of another organ and causes a new virus multiplication in a second "organ" phase. Phase 1 does change directly into phase 2, but there is often a symptomless interval between them, the duration varying markedly from sickness to sickness. Thus, the "remission" (symptomless interval) in yellow fever lasts only one day on the average, whereas in TBE the interval is mostly a week. With localized organ manifestations there are essentially only three preferred places, i.e., the CNS, liver, and mesenchyma, so that we know of several kinds of disease systems accordingly, such as meningoencephalitis, hepatitis, and dengue fever.

In phase 2 the virus clings to the affected organ, concentrating in the sensitive cells when it is destroyed. Flow of the virus into the blood vessel can no longer be traced since at this time, at least in the case of CNS infections, neutralizing antibodies are already present in the serum, although the virus can be found in the organ itself.

Thus, there is no clear evidence to permit a pathological-histological differential diagnosis of most of the CNS infections caused by arboviruses.

Animal experiments with arboviruses do not provide a proper basis for making correct comparisons between the behavior of the virus in humans and in animals so that study of the pathogenesis of human infection in concrete fashion is dependent wholly on autopsy findings which, however, due to the slight lethality of the arboviruses, are too meager to furnish a complete picture of the dynamics of this infection. It is still unexplained why a phase 2 develops at all following phase 1 in particular cases. The only demonstrable constant in arboviruses is organ tropism which, to be sure, does not correlate with the antigen structure of the viruses. Perhaps we should think in this connection of the variable affinity of individual viruses for certain phospholipids which as constituents of the cell surface could influence the adsorption of a virus.

The clinical picture of the arboviruses is so varied that these differences are to be related not only to the "tropism" of the viruses but also to the range of variation within a disease system as a whole. Without going into details (see the individual chapters), a basic distinction must be made between the course of the two phases according to the pathogenesis. Phase 1 includes all transitional forms from the clinicallyinapparent→ mild to moderate (up to 38° C) fever → high fever. In addition to fever, along with general complaints like headaches and joint pain there can appear catarrhal phenomena (conjunctivitis, pharyngitis, bronchitis) and, in very severe cases, hemorrhages as a result of hemorrhagic diathesis. Phase 1 generally lasts only a few days, but as much as 10 days in severe cases of HF.

Phase 2 is characterized chiefly by organ involvement so that the general picture of the disease with respect to length, degree of severity, and prognosis is determined thereby. Apart from these organ-conditioned circumstances, an exanthema can develop in this phase as an expression of vascular injury (dengue, Chikungunya, West Nile) or, in especially severe cases, a hemorrhagic diathesis, e.g., in yellow fever or EEE, wherein the latter ends as a hemorrhagic encephalomyelitis.

E. Immunobiology

An infection of a vertebrate, unlike that of an anthropod, invariably results in the formation of specific antibodies which can be found in the serum by the appropriate diagnostic technique. This antibody formation goes back to the sensitization of the organism during the viremic phase (= phase 1) when the neutralizing and hemagglutinating antibodies can be generally detected after about 7-10 days.

In those biphasic diseases, e.g., CNS infections, in which more than a week elapses between the beginning of the viremia (= phase 1) and beginning of the illness (= phase 2), these antibodies are always present before the onset of phase 2. In TBE the interval averages 12 days. This phenomenon is crucial for diagnosis because a titer increase in the NT and HIT, not just a conversion in these seroreactions, is to be expected in the course of such diseases.

On the other hand, the complement-fixing antibodies are not always evident at the onset of phase 2 so that a conversion is to be expected and, consequently, a fresh infection demonstrated rather easily. A peculiarity of antibody formation in the arboviruses is that the specificity of the antibodies decreases with the duration of sensitization (be it in the course of an infection or during an active immunization with inactivated virus) while antibodies are increasingly formed against other, mostly closely related, strains of the same group. This can be very easily traced in the HIT and CFR because this overlapping within a group is here manifested very clearly. A broad antibody spectrum can also be achieved by immunization with a pair of strains from one group, whereupon cross reactions with almost all strains of the same group can then appear.

This phenomenon operates, of course, to the disadvantage of an investigator when he is seeking specific antibodies in a region in which several strains of the same group are found side by side so that he is unable to make any reliable statements without quantitative NT. A population, on the other hand, is benefited because with every new infection (or immunization) the immunity spectrum is broadened to embrace other heterologous antigens (cf. Prophylaxis).

Serological Investigational Methods

(a) Neutralization Test (NT)

The infectiousness of a virus is neutralized in the NT. Intracerebral administration of a serum-virus mixture to mice is the standard test. The sensitivity of the test to the presence of antibodies is clearly dependent on the inoculation route (i.p. is more sensitive than i.c., the baby mouse is more sensitive than the adult mouse). Moreover, it has been shown that fresh normal rhesus serum contains a factor (accessory or labile factor) that increases sensitivity to the presence of antibodies. With respect to the NT in mice, Theiler prefers the average survival time to the survival rate in reaching conclusions.

In addition, tissue cultures have recently come into use. They have proven to be a very sensitive indicator and more efficient than the mouse test. The serum-virus mixture can be transferred to a dense medium or simultaneously sown with a relatively low cell content (15,000-30,000 ml) in test tubes[95, 96]. It has to be determined

in each individual case whether suitable cells are available in which an increase through the CPE or hemadsorption [26] can be easily read.

A special type of NT involves the use of the "plaque technique" whereby one can distinguish between a: (1) plaque-neutralization test [48] (virus-serum fixation in vivo before inoculation of the medium); (2) plaque-reduction test (addition of serum to the agar layer after inoculation of the medium); (3) plaque-inhibition test [136]. The plaque or plaque-reduction test is very sensitive and suitable for subtle quantitative investigations. The plaque-inhibition test is quite similar to that used for antibiotics against bacteria because here too the diameter of inhibition of the CPE of a tissue culture is measured under an agar layer. The inhibition areola results from the diffusion of an antibody-containing serum or other inhibiting substance, e.g., interferon [135], through the agar layer on the infected medium.

(b) Hemagglutination-Inhibition Test (HIT)

This test is now performed everywhere in a standard manner [39]. It is an excellent procedure for infection inquiries (survey) because as a result of agglutination-associations within individual groups antibodies can be detected in a population even with non-homologous antigens. The HIT is quantitatively prepared so that there is a linear correlation between agglutination and antibody concentration.

(c) Complement-Fixation Reaction (CFR)

This reaction is used to detect fresh infections (also to identify freshly isolated strains). Fulton and Dumbell's microdrop method [53] is employed, with the reaction started in the usual way (with serum dilutions against constant quantities of Ag and complement) according to Casals et al. [33]. Plastic plates (the Linbro Chemical Company's disposo-trays) are suitable for this purpose. The bowls are so shaped that the reaction is clearly readable despite the small total volume of about 0.15 ml.

F. Epidemiology

The most striking characteristic of arboviruses is the peculiar cycle of the virus in nature in which arthropods, i.e., as far as we know, ticks or blood-sucking mosquitoes, are invariably included as vectors. This produces a characteristic rhythm in the chain of infection that varies with the habits of the affected animal host, individual species of the arthropod, and differences in climate (tropics, temperate zone). This is also reflected in the seasonal pattern of many infections (early-summer meningoencephalitis [ESME] and autumn encephalitis [JBE]).

1. Characteristics of the Arthropods

Up to now arboviruses have been isolated from Culicidae, Ceratopogonidae, Simuliidae, Psychodidae (Phlebotominae), various Brachycera, Ixodidae, and some other ticks (Gamasina). Among these arthropods the vector function, which affects the arboviruses of man, is thus far sure only in the Culicidae, Phlebotominae (cf. pappataci fever), and Ixodidae through evidence of multiplication capacity of the virus after ingestion with the blood meal.

(a) Mosquitoes

The Culicidae consist of several subfamilies (e.g., Anophelinae, Culicinae) and numerous genera (e.g., Anopheles, Aedes, Mansonia, Culex, Psosophora, Haemagogus, Culiseta). These genera differ not only in morphological characteristics, but above all in their behavior in the environment, host specificity, life span, manner of hibernation, and geographic distribution.

Ingestion of the virus occurs with the blood meal when a critical minimum quantity must be present in the blood ingested. The virus then multiplies in the tissue and is eliminated in the salivary gland. The interval between virus ingestion and elimination is known as the "extrinsic incubation period". The duration of this interval is dependent primarily on the temperature and relative humidity, secondarily on the virus-transmitting species. The female mosquitoes remain infectious for life and can infect the host during all additional blood meals. Transovarial transmission of the virus to the F_1 generation has not yet been observed. The development is holometabolous, i.e., over the well demarcated stages of egg, larva, pupa, and imago. Culicid larvae are aquatic (stagnant water) and feed on plankton. The life span of the mosquitoes (imagines) varies greatly with the ecology and biology of the individual species and is several weeks. It may extend to 8 months in case of overwintering (e.g., Culex, Theobaldia). Apart from the species, climatic factors, especially in the temperate zone, manner of hibernation, whether as egg, larva, or imago, play an important role; also whether the mosquitoes move freely about in nature or predominantly within the range of human settlements (cellers, stables). The radius of action of a mosquito likewise varies from species to species, but generally does not extend more than 20 km, although it can be borne to great distances by passive locomotion (wind).

Due to the great radius of action and capacity of the females (the males never bite) for repeated meals, a frequent exchange of host is possible so that during the warm season, even in the temperate zone, a virus infection can spread quickly among human beings, domestic and wild animals. Since infants and children are attacked, infection of the population occurs mostly at an early age. The mosquitoes swarm mostly at dusk because their activity is largely dependent on the temperature and relative humidity.

(b) Ticks

Arboviruses are transmitted by representatives of the Argasidae and Ixodidae. They exhibit an abundance of specific morphological features, but can be considered together in view of their function as vectors.

Unlike the blood-sucking mosquitoes, ticks can suck blood as early as the larval and nymphal stages and thus transmit virus in all stages. Another characteristic of the ticks is that they have only one host in each stage, that is, the next blood meal occurs only in the next higher stage, except for the adult female Argasidae, which suck blood several times. The blood-sucking act itself lasts a few days. In principle, there are only 3 main stages (larva, nymph, adult), but the Argasidae have several nymphal stages (1st, 2nd, 3rd instars). Hence, the ticks do not have the so-called "extrinsic incubation period", but the virus multiplies during metamorphosis in the various organs and is excreted with the feces and in the next higher stage with the saliva. In addition, the virus can also be transmitted to the next generation transovarially. It is still uncertain whether this phenomenon of transovarial transmission is related to the fact that tick eggs are covered by a single membrane while mosquito eggs have three membranes [181]. Transovarial transmission has been repeatedly demonstrated in the laboratory (cf. TBE), with confirmation seen in the fact that under natural conditions the virus can always be isolated from hungry larvae. The percentage of transovarial transmission of the virus to the F_1 generation, however, would have to be very small, being estimated at about 5% of the number of eggs deposited. But it is not enough to maintain the virus in a focus.

Ticks live much longer than mosquitoes, often several years, if for partly still unknown reasons the sequence of stages does not follow regularly but under certain circumstances is interrupted for an entire year. However, under optimal conditions in the laboratory, all the stages of a three-host Ixodes ricinus can be traced within 8 months.

Since ticks live freely in nature and have no connection with human civilization, unlike mosquitoes, man functions as a blood donor only when he happens to be in their territory. A direct consequence of this is that a population never becomes as infected as it does through mosquitoes. Living habits and occupational patterns of the autochthonous population are of decisive significance. A striking fact is that infection occurs at a much later age than that caused by mosquitoes and, therefore, ticks are not responsible for the transmission of "children's diseases". It is also possible that this "late" first infection is responsible for the particular clinical course that often proceeds with (apparently age-conditioned) complications (cf. TBE). The "active" radius of action is so slight that it can be virtually ignored. Nevertheless, ticks can be borne considerable

distances while sucking on v[illegible] (e.g., birds flying from continent to continent). The host spectrum corresponds to the three stages, for the larvae attack only small animals (chiefly rodents) and the adults always attack larger animals, while the nymphs show no significant preference. This depends partly on the behavior of the animals, partly on the inability of the larvae to perforate the thick stratum corneum of a large animal with their mouth parts. Man, therefore, is bitten only by nymphs and female adults without being aware of it. It is only some time later (12 to 24 hours) that he feels an itching around the locally reddened puncture site with the still sucking tick. Many ticks, especially the Dermacentor species, give off a neurotropic toxin with the puncture which can result in "tick paralysis" in humans and animals. This neurotoxin has nothing in common with neurotropic viruses.

2. Characteristics of the Animal Host

The vertebrates bitten by arthropods and infected as a result develop a viremia after an incubation period of several days to a maximum of a week and in this stage become a source of infection for all biting arthropods at this time. Lactating animals in the viremic stage eliminate the virus with the milk. The duration of the viremia varies from vertebrate to vertebrate, but generally is no more than a week. However, there are reports on CTF virus [27, 26] causing a viremia lasting up to 50 days in porcupines and some other rodents. Also, a viremic stage can be artificially prolonged [193].

A viremia in a natural animal host proceeds without clinically apparent disease and it is followed by the formation of neutralizing antibodies without a second phase occurring after a short interval. If this natural cycle changes or if the natural infection spectrum is broadened into an "artificial" infection spectrum (according to Doerr), symptoms of disease will be observed in animals (e.g., equine encephalitis) and human beings (all the known arboviruses). Chains of infection in which at least part of the vertebrates needed to maintain the natural cycle falls ill in the typical way (e.g., sheep with louping ill) are exceptions.

In the formation of a focus, vertebrates function as the virus reservoir. Should this focus be in the tropics, a continuous chain of infection with arthropods is produced because no climatic fluctuations influence the multiplication of the arthropods and vertebrates. In the temperate zones, however, the question of virus overwintering is still unanswered. Apparently the virus overwinters on the spot in hibernating or poikilothermic animals or in the ticks (transovarial transmission). The possibility that it can be introduced into new places by migratory birds should not be ignored.

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We must distinguish in the focus between vertebrates of the basic natural cycle and those vertebrates which, like human beings, are infected only facultatively, and are not absolutely essential for maintenance of the cycle. They can, to be sure, contribute to the spreading of the virus, but they cannot by themselves along with the arthropods maintain the cycle. Consequently, a precise analysis of the significance of the individual vertebrates in a focus is very difficult.

3. The Focus

A natural focus of infection requires a host reservoir and vectors, on one hand, and an adequate density of vertebrates and vectors, on the other, to endure. The loss rate is so high that close and intense contact between vertebrate and arthropod, at least in certain seasons, is needed if the virus losses, especially in the temperate zones during the winter months, are to be halted. This also promotes infection through a viremia of maximum duration and through rapid succession of generations of the vertebrates serving as the reservoir.

Apart from the decimation of vertebrates and arthropods by their natural enemies, climatic factors, etc., the antibody formation of the once infected vertebrates operates as a counter-mechanism of infection. Even if we disregard Benda's experimental [20] but not otherwise confirmed observation of the neutralization of a virus with antibodies in an arthropod following a new blood meal, the chain of infection is broken in the case of attack by an infectious arthropod on an already immunized vertebrate. Since viremia is brief and it occurs once in the lifetime of a vertebrate while the neutralizing antibodies remain permanently, with increasing life-span of the vertebrate, the chance of virus spread diminishes accordingly.

In an extreme case, a focus can also be extinguished if it is not reinfected from the outside. There is evidence in connection with human diseases, e.g., the disappearance of "Australian X disease", which support such considerations.

4. Natural Cycles

From the epidemiological standpoint, three different kinds of cycles can be distinguished in nature:

(a) Primitive Cycle of a Germinal Chain of Infection

→ Tick (P-gen.) → transovarial → Tick (F_1-gen.) → transovarial →

Theoretically, this cycle must be postulated as possible because transovarial transmission of arboviruses by ticks has been demonstrated experimentally and under natural conditions. It is debatable whether this cycle can ever exist by itself [161]; in any case it is judged

today only as a subcycle as far as the flavivirus arboviruses are concerned. The existence of such a cycle, however, enables us to derive therefrom the origin at least of the tick-borne arboviruses.

If we start from the fact that arboviruses multiply in the cells of ticks without causing, however, pathological changes, we must postulate a close relationship between these cells and the viruses. It must be closer than in the case of vertebrate cells which are either destroyed or react by producing antibodies. We could conclude from this that at some time in the course of evolution part of the nucleic acid from the cell of a tick split off and became independent, transmitted transovarially, for instance, adapted in time through passages in vertebrates as well, and assumed the form of the arboviruses known to us today [181].

(b) Cycle with a Homogeneous Chain of Infection

→ arthropod A → vertebrate 1 → arthropod A → vertebrate 1 → arthropod A →

This homogeneous chain of infection is the basis of every focus in which the vertebrate is absolutely essential for maintenance of the cycle in the focus. A special form of this cycle with the inclusion of man produces yellow fever:

→ Aedes → man → Aedes → man

A cycle with inclusion of man as reservoir has no priority, but in the present case it gives rise to a secondary transformation of the natural infection spectrum (→ monkey → Haemagogus → monkey → Haemagogus →) as a result of change in environmental conditions.

(c) Cycle with a Heterogeneous Chain of Infection

→ arthropod A → vertebrate 1 → arthropod B → vertebrate 2

This heterogeneous chain of infection is very common in nature because within a focus mostly vertebrates are infected by bites. Here various species of anthropods present at the same time, especially as concerns mosquito-borne viruses, can transmit the virus. It is very difficult to decide which vertebrates and arthropods are absolutely essential to maintain the focus. On one hand, arthropods infect various vertebrates which are meaningless for the cycle and which function only as blind terminal or subordinate members of a chain of infection, as happens, for example, to man with most arboviruses. On the other hand, blood-sucking arthropods on viremic vertebrates become infected without the virus being able to spread further (fleas and lice). Therefore, during a field investigation one must not draw hasty conclusions on the basis of virus isolation and/or presence of antibodies.

5. [illegible]

Arboviruses are generally [illegible] circulate in their natural cycles, with the infection of man (or susceptible animals) playing a minor role.

Infection of vertebrates depends on their habits and on the behavior of the vectors. In the case of viruses transmitted by mosquitoes, the rate of infection is high even among infants, especially in the tropics. As far as tick-borne viruses are concerned, much depends on the extent and time of man's contact with the indigenous ticks. In general, infection occurs much later so that the rate is considerably lower. In most cases, however, the theoretically endemic character of the infection is preserved. But if this situation of an endemic focus is changed, the virus may also spread epidemically: (1) spread of the virus from the focus to a non-immunized population (jungle yellow fever → urban yellow fever); (2) massive immigration by non-immunized vertebrates into a focus (epidemic outbreaks of yellow fever among colonists, builders of the Panama Canal, etc.); (3) consumption of nonboiled and non-pasteurized milk of viremic vertebrates (epidemic of early-summer meningoencephalitis in Roznava [24].

6. Spread of Viruses to Alien Territories

This particular matter is given considerable attention not only for hygienic reasons but also for theoretical considerations related to the potential development of a new focus. Viruses can be borne great distances by migratory birds, infected human travelers, and arthropods in ships, airplanes, etc. However, such viruses cannot create a new focus without the existence of the aforementioned conditions pertaining to vertebrates and vectors. Allowance must also be made for the possibility that other antigenically related viruses are predominant in the new place and that they have already resulted in immunization of the vertebrates. This could interfere with the further spread of a newly imported infection. The phenomenon is often cited to explain why in Egypt the autochthonous population infected with West Nile is not susceptible to yellow fever, even though the other prerequisite for a yellow-fever focus (climate, vector) are present.

Another possibility is that migratory birds supply new virus or infected arthropods every spring for the seasonal arboviroses that occur in the temperate zone. This is considered to be very likely in the case of foci of WEE and EEE in the United States.

There is also some significance in the spread of virus through human beings during the incubation period and through arthropods carried in airplanes. The yellow-fever virus, originally indigenous to Africa, might well have reached America via infected mosquitoes transported in boats. Since the possibility of spread is greatly increased by modern air travel, the World Health Organization has made recommendations to disinfect planes used in international traffic [211].

3. Prophylaxis

Measures to control the spread of [illegible] arbovirus infection can be directed both against the vertebrates as host of the reservoir and against the arthropods as vector. In addition, specific prophylaxis of human beings and endangered animals can also be achieved by active and passive immunization.

All attempts to destroy or at least to reduce the virus reservoir to such an extent that a natural cycle can no longer be maintained fail mostly because of the lack of knowledge as to which species of animal is to be regarded as the reservoir. Moreover, it is very difficult, if not impossible, nowadays successfully to control, for example, certain birds or rodents in a focus.

Human intervention in a focus seems rather to achieve an effect opposite to that intended when, for example, the natural "enemies" (predatory birds or animals) are eliminated, thus enabling the animals serving as the virus reservoir to multiply beyond their normal limits. On the other hand, arthropod control as a means of eliminating a focus is a time-tested method that has proven to be very effective, especially in eradicating yellow fever from American port cities. The development of modern insecticides and acaricides has given fresh impetus to this approach and it has made it possible to treat much larger areas than before. The Russians in numerous field expeditions to combat the widely diffused ticks have been particularly active in this respect. However, just how lasting the effects can be is problematical because unlike the arthropods which have adapted to man and his domestic animals and which live very close to him, the arthropods which circulate freely in nature, especially the ticks, are far more difficult to get at than mosquitoes, apart from their almost unlimited range.

In addition to these general control measures, planned immunoprophylaxis is highly important for man and his domestic animals. Active immunization has been successfully used for many years - with vaccines containing virus inactivated by formaldehyde or with vaccines containing attenuated strains.

The purpose of passive immunization is to provide immediate aid for a victim of a laboratory infection, but it can scarcely be considered for general use. Hyperimmune sera or hyperimmune globulins against TBE virus have been produced and suitably tested for this purpose.

1. Tick-[illegible] [illegible] Complex

The TBE complex consists of [illegible] virus strains (cf. tables) which are transmitted by ticks and are so closely related in antigen structure that they cannot be distinguished from one another without the help of absorbed sera or cross precipitation in agar gel [41]. The term "encephalitis" shall therefore be taken to mean that most strains of this complex are capable of causing a disease of the central nervous system (CNS) in man and animals. Typical diseases include:

1. TBE virus

   (a) RSSE/subtype: Russian spring-summer encephalitis
   (b) CEE/subtype: central European summer meningoencephalitis

2. LI type virus: louping ill of sheep

Here also belong two virus strains that were isolated from the brains of persons who died of meningoencephalitis without, however, their being held responsible (thus far) for a CNS disease endemic in the region.

3. Powassan type virus (Canada) [101]

4. Negishi type virus [127]

5. Langat TP 21 type virus

This virus was isolated in 1956 from Ixodes granulatus in Malaya [57]. Ixodes granulatus normally does not attack human beings, but neutralizing antibodies against this virus have been occasionally found in the native population of Malaya [59a]. The way the virus spreads is still unclear. The virus strain itself has only slight neurovirulence for man and animals and it is used as an attenuated vaccinal strain for animals (cf. Louping Ill).

The next two types produce in man the clinical symptoms of a hemorrhagic fever:

6. Omsk type virus (with subtypes I and II): Omsk hemorrhagic fever, Central Siberia (cf. Hemorrhagic Fevers)

7. KFD type virus: Kyasanur forest disease, India (cf. Hemorrhagic Fevers)

2. [illegible] Encephalitis

Synonyms: Russian spring-summer encephalitis, Far East forest encephalitis, taiga encephalitis

According to Russian data [130], many cases of human meningoencephalitis have been observed in the Far East since 1932. A field expedition started in 1937 under the direction of Silber and concluded in 1939 succeeded in isolating a variety of virus strains from humans, ticks, and rodents and in elucidating the infection cycle [213].

In 1941 Smorodintsev et al. [175a] reported on successful attempts at active immunization of human beings with formalized vaccines prepared from infected mouse brains. In 1943 and 1944 Casals and Webster [34, 35] discovered the antigen relationship with louping ill.

According to Pawlowsky, this virus is transmitted chiefly by Ixodes persulcatus and, possibly, by Haemaphysalis concinna and Dermacentor silvarum. In all three cases it is a matter of three-host ticks in which the virus not only survives the metamorphosis but is transmitted transovarially [130].

Ixodes persulcatus exhibits a peak of activity early in the summer and correlates well with the seasonal pattern of human diseases [174]. On the other hand, "autumnal encephalitis", which also occurs in the Far East, is caused by JBE virus and transmitted by mosquitoes [176].

The actual geographic distribution of the disease in the Far East and in Siberia has nowhere been precisely mentioned by the Russian authors. It seems, however, to correspond to the range of Ixodes perculcatus, not much beyond the 60th parallel to the north [124, 175].

The most striking characteristic of the disease, as noted by the Russian authors, is the severe clinical course with a high rate of paralysis and 30-40% fatality. It is not known whether the high death rate is due to the increased neurovirulence of the virus in Ixodes persulcatus or to a special susceptibility of the native population or to the exclusion of some (especially the mild) cases of the disease from the statistical data.

Studies on the pathogenicity of this virus have shown only that this Far Eastern strain is more likely to cause paresis and paralysis in sheep and monkeys than are the viral strains of the CEE subtype [214, 215]. At any rate, the unusually severe course of the disease in man seems to have been instrumental in intensifying efforts actively to immunize the exposed population. These efforts were apparently successful [37, 39], despite the fact that the vaccines originally prepared from mouse brains (today from tissue cultures, [93]) led to complications.

In view of the large-scale use of acaricides to control ticks in the rural areas, much useful experience has been gained and, according to the data, the morbidity rate lowered [50, 60, 114, 122].

3. Louping Ill

Synonyms: spring sickness or staggers in sheep

This disease is described in detail in the chapter "Human Infections Through Animal-Pathogenic Viruses" by M. Mussgay

4. Early-Summer Meningoencephalitis (ESME)

Synonyms: Central European encephalitis (CNE), tick-borne encephalitis, Kumlinge disease (Finland), biphasic meningoencephalitis [116]

The earliest clinical and epidemiological observations on the spread of ESME in Europe date back to Schneider, who found in 1927 in Neunkirchen (Lower Austria) a number of benign, mostly meningitic forms of CNS diseases [183]. In a monograph published in 1931, he described his first observations and experiences with 66 patients whose disease he regarded as a new, then unknown infection but sui generis [184]. Borrowing from Wallgren, he called the disease "meningitis serosa", today chiefly diagnosed as meningoencephalitis. At that time there were cases with a severe course, especially with paralysis, but their etiology was not recognized because at autopsy only the lesions typical of poliomyelitis were found in the region of the anterior horn of spinal cord. Hence, all these cases were interpreted as poliomyelitis or, if the clinical course was unusual, as atypical poliomyelitis. All attempts at isolation of the virus failed. There are still some observations on such diseases of the CNS which go back to that time and may be relevant, e.g., the cases observed in the area of Szeged [23] and Kaschau [50].

The etiology of the disease was accurately determined in Central Europe after the war when in 1948 the causative agent was isolated in Czechoslovakia for the first time and systematic serological studies were carried out on its distribution [72]. During the next 10 years the infection was also discovered in Finland [126], Sweden [180], Bornhom, Denmark [52a], East Prussia [175], Poland [142], East Germany [171], Hungary [91, 114a], and Jugoslovakia [82]. In Austria, the virus was isolated in Styria [194] and in the region of Neunkirchen [117]. Serological examinations of the numerous patients of the past 30 years revealed that the cases observed by Schneider and regarded as an infection sui generis should actually be diagnosed as ESME. It was clear proof that this disease was not imported from the East for the first time during the war, that it had already been present, at least in the Neunkirchen area [118, 119].

After the war (1949-1955) a new form of the disease called "biphasic meningoencephalitis" (according to Smorodintsev) was found in European Russia. In this very long disease the virus is transmitted to man through drinking raw goat's milk (hence also "biphasic milk fever") (Chumakov). It involves a fundamentally different kind of virus cycle in nature but merely the inclusion of lactating goats (infected by ticks) which eliminate the virus with the milk. If there are many goats and a high consumption of their milk, the situation can be quite significant in the transmission of the virus to human beings. According to Clarke [40], this virus is indistinguishable in antigen structure from the CBE type CBE virus, which is found in European Russia and transmitted to man by ticks.

(a) Physical, Chemical, and Biological Characteristics

Electron-optical studies have shown that viruses in HeLa cells are regularly 25 mμ in size, round, with a thick inner body and clear outer zone [86]. The particle weight is estimated at 10 million [177]. Purified extracellular virus has a diameter of about 30 mμ in the electron microscope. Estimates of the sedimentation constant in the ultracentrifuge, however, indicate a particle weight of 20-25 million. It is thus conceivable that the virus increases in size as a result of an excretion process.

The infectious virus particle is relatively stable at 4° C, especially in the presence of at least 10% serum, and it survives in milk and butter up to two months [64]. On the other hand, it is inactivated within 10 seconds at 85° [65]. According to Gresikova-Kohutova [63], it is still stable in a pH range of 2.75-11.95 with an optimum of 7.6-8.2 so that it is detectable in sour milk (pH 4-5) even after 24 hours at 4° C. Albumin-decomposing enzymes (trypsin, chymotrypsin, papain) attack [36] and inactivate the virus (as well as the other group B viruses) but not the group A viruses. The virus also loses its infectiousness as a result of the usual chemical inactivation procedures (formaldehyde, β-propiolactone). A virus suspension can be purified by means of hydrocarbons [177], through adsorption on calcium phosphate [58], and with protamine sulfate [39], possibly also through adsorption on erythrocytes and subsequent elution [153].

HA produced according to Clarke and Casals [39] and the infectious virus particle at pH 6.2-6.8 have the capacity to agglutinate goose and rooster erythrocytes; Salminen [153] succeeded in again eluting the virus at pH 9.0 from the erythrocytes. However, this hemagglutination is highly sensitive and it can be inhibited not only specifically by hemagglutination-inhibiting antibodies but also by lipids appearing normally in the serum. The latter can be removed from the serum through adsorption on kaolin and, best of all, through treatment with acetone [39]. It appears to be a matter of a complex of free cholesterol with the most important serum phosphatides or a complex of free cholesterol with free fatty acids, which presumably adsorb the

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virus is the form of an [illegible] the lipids on the surface of an erythrocyte [illegible]. The inhibition of hemagglutination by viruses of [illegible] distinguished by Porterfield and Rowe [137] could not, however, be confirmed in detail by Salminen [157].

Intracellular virus multiplication can occur in numerous tissue cultures, but a CPE is not always observable. This can be ascribed either to the peculiar nature of the individual virus strain or to the specific behavior of the tissue culture, especially of the permanent Detroit-6 and HeLa strains [91]. Virus synthesis can be traced in time and place by the addition of fluorescein-labeled antibodies [89]. Within eight hours the virus antigen and elementary corpuscles appear in the region of the Golgi apparatus of the HeLa cell [87].

Suckling mice (also, if necessary, larger mice up to 20 g) are used as sensitive experimental animals, whereas chick embryos, chicks, suckling rats, hamsters, and monkeys are no longer required. The virus, of course, attacks domesticated animals like cows, sheep, and goats, which develop a viremia and eliminate the virus with their milk. On the other hand, an encephalitis does not follow peripheral inoculation.

Among the arthropods, culicids became infected by feeding on viremic mice. The virus could be detected, in general, after 1-2 days in the culicids [129, 130, 139, 173], although they were not eliminated by the mosquitoes. Under experimental conditions Ixodes ricinus [20, 21, 99] and Ixodes hexagonus [166, 193] were allowed to become infected in the natural way. Here the virus could be re-isolated directly after a blood meal from the feces and also from the next higher stage (by pulverizing the nymphs or adults). However, only Benda [21], Streissle [180], and Rehacek [146] successfully achieved transovarial transmission. Virus multiplication in ticks appears to be limited to the Ixodidae. It could not be produced in the Argasidae [85, 187].

Other arthropods too, e.g., fleas, can be infected by sucking without, however, virus multiplication resulting [145]. On the other hand, Jettmar [80] showed in naturally infected triatomas that while they harbor the virus all their lives, they do not eliminate it with saliva so that they cannot be considered vectors.

(b) Clinical Symptoms, Pathohistology, Immunology

The first phase of the disease (viremia) sets in after an incubation period of 7-14 days. It is accompanied, in general, by a fever of up to 38° C, vague headache, pain in the spine, joints, and muscles along with inflammatory changes in the eye, nose, and throat region. These symptoms subside after a few days, whereupon an asymptomatic interval follows, lasting until the beginning of the second phase.

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This first phase is not noted retrospectively by some 60% of the patients when they come to the physician in the second phase and are questioned. Frequently the complaints were so minor that they continued to work and did not consult a physician. Accurate observations on the course of phase 1 are possible only when the patients are admitted to a hospital with unusual severe complaints, thus permitting both phases to be closely followed, or in the case of laboratory infections where phase 1 can be traced from the very beginning (Fig. 2). Otherwise, it is also practically impossible in the spring in an endemic area either to detect all the common infections of this kind or conscientiously to follow them up clinically and virologically (isolation of virus from blood) to permit epidemiological evaluation. Moreover, morbidity and infection rate of the population in an endemic region are too low.

Phase 2 sets in acutely after a symptomless interval of about 8 days (or about 12 days after the beginning of phase 1). The clinical course shows a striking age dependence (cf. table). Whereas the meningitic form is predominant up to the age of 40, encephalitis is predominant from 40 to 60, and the paralytic components after 60.

The meningeal form of the disease apparently does not involve the parenchyma of the CNS and it presents no unusual features, but it subsides like all the "serous" virus-caused meningitides after 3-5 days, with complete restitutio ad integrum. The clinical diagnosis of "serous meningitis" can then be based on the course (neck stiffness, fever over 39° C) and spinal fluid (cell count to 500/3 with lymphocytes predominating and albumin values increasing as the disease persists). This picture is characteristic, but not specific for ESME.

The encephalitic form (meningoencephalitis) follows a diverse course. It is generally combined with meningitis. Besides neck stiffness, frequently only twitching of the muscles of the face, tongue, and extremities, vertigo, disorders of sensibility, drowsiness, impaired reflexes, etc. indicate pathological changes in the encephalon. There are also malignant, occasionally fatal encephalitides in which symptoms like paralysis of the eye muscles, speech disturbances, fascial and other cerebral nerve pareses, unconsciousness, and psychoses dominate in the acute stage. The duration of the acute stage and the possible sequelae vary accordingly. "Late paralysis" is a peculiar development. It is found mostly in the upper extremities 8-10 days after the onset of fever. It is frequently associated with disorders of sensibility in the affected parts, but not with elevated temperature. Recovery is rapid without atrophy or residual lameness. Presumably it is a matter of a neuroallergic reaction to an acute infection of the CNS. An etiological diagnosis cannot be based on the many-sidedness of the clinical picture.

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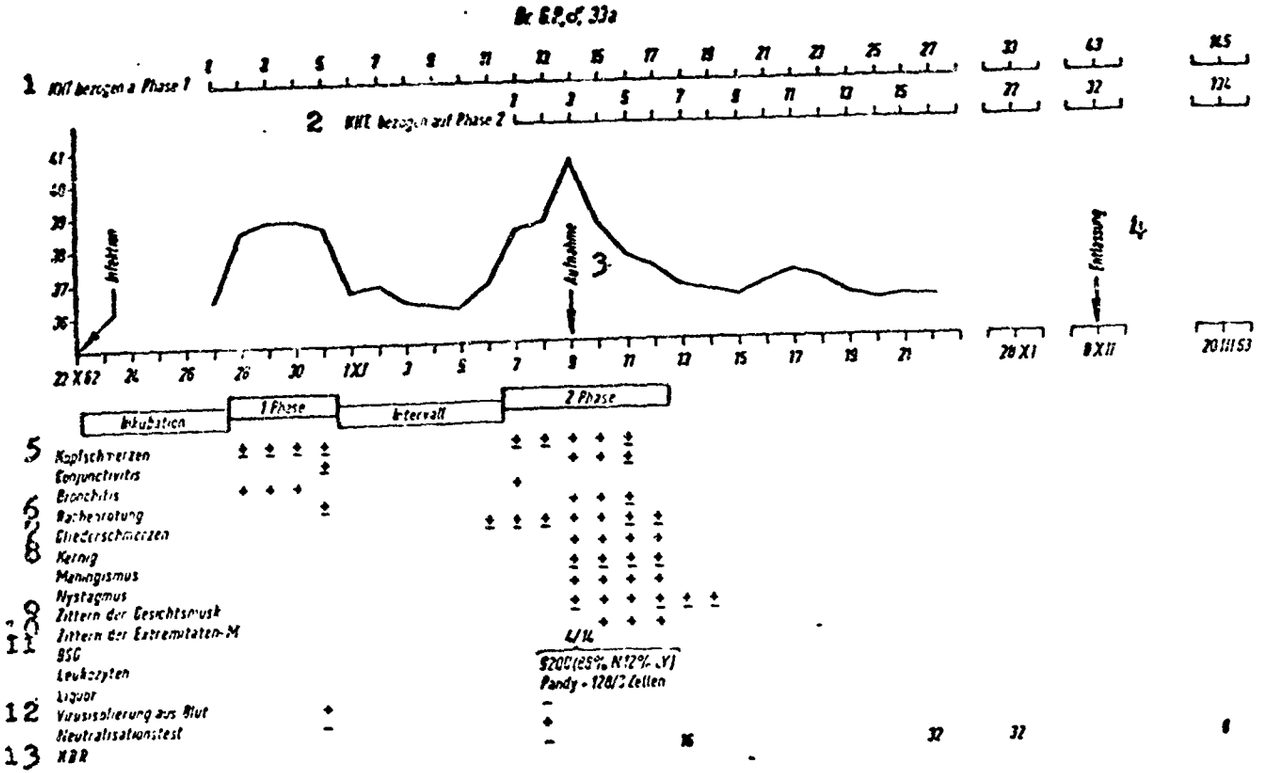


Fig. 2. Clinical course of a laboratory infection with EMC virus

1 - days of sickness in relation to phase 1; 2 - days of sickness in relation to phase 2; 3 - admission; 4 - discharge; 5 - headache; 6 - sore throat; 7 - joint pain; 8 - Kernig's sign; 9 - tremor of facial muscles; 10 - tremor of extremities; 11 - BSG (?); 12 - isolation of virus from blood; 13 - complement-fixation reaction

The paralytic form is [illegible] by flaccid paralysis, with [illegible] for the muscles of the shoulder portion). Besides this [illegible] paralysis and ascending forms [illegible] forms have clinical indications [illegible] ESME. But even for the experienced [illegible] possible to definitely rule out [illegible] are not attacked, the paresis [illegible] half a year later show.

Table 1

Distribution of all CNS Infections by Age in an Endemic Region of ESME, 1956-1962 (Neunkirchen/Austria) (after Krausler)

2 Altersstufen	1 FSME: 3 meningitisch	4 enzephalitisch	5 paralytisch	6 andere Infektionen: 4 meningitisch	5 enzephalytisch	6 paralytisch
1—10	11	4	—	18	8	9
11—20	11	15	2	23	8	10
21—30	9	15	2	15	8	3
31—40	9	16	—	10	5	3
41—50	11	32	6	—	4	—
51—60	5	19	3	5	5	1
61—70	2	8	4	—	4	1
71—80	—	3	1	—	1	—
81—90	—	—	1	—	—	—
total	58	112	17	71	43	27

7 Gemäß dieser Aufstellung beträgt das Durchschnittsalter für FSME 37,3 Jahre und für alle anderen Infektionen des ZNS 23,1 Jahre; der Altersunterschied ist statistisch sehr signifikant ($t = 7,0$, $p < 0,01$).

1 - ESME; 2 - age classes; 3 - meningitic; 4 - encephalitis; 5 - paralytic; 6 - other infections; 7 - the average age for ESME is 37.3 years, for all other CNS infections 23.1 years; the age difference is statistically very significant ($t=7.0$, $p<0.01$)

Besides residual lameness and atrophy after the paralytic form of ESME, about 10% of the patients also complain of autonomic dystonia, especially with persistent headaches. Now and then parkinsonism, diabetes insipidus, schizophrenic psychoses, and epileptiform states also occur. The latter were observed back in 1880 by Kojevnikov as the sequela of encephalitis in the Far East, where this could possibly indicate the existence of the infection.

There is no specific therapy; the administration of antibodies in a manifest phase 2 has proven to be unsuccessful. The basis for all the clinical symptoms are the organ changes, which can be objectified by pathohistological investigations. Not only are individual

parts of the CNS investigated, but the CNS is fixed in toto and through a suitable work-up the topical distribution of the lesions is determined.

Seitelberger and Jellinger [163], Jellinger and Kovac [76], and Grinschgl, Kovac, and Seitelberger [67] reported on such systematic investigations, which were briefly summarized by Seitelberger [164] and Jellinger and Seitelberger [77]. A diagram derived from a series of verified fatal cases plus consideration of the histological features of the encephalitic syndrome made it possible to set up morphological criteria for distinguishing the disease from poliomyelitis, as conjectured by Bednar [19], Kornyey [84], and Joba [81] on the basis of the distinct involvement of the cerebellum. On the other hand, no theoretical differences from other arbovirus infections of the CNS emerged from the nature and site of the CNS inflammatory process. This is highly important because in spite of a certain qualitative difference between ESME and poliomyelitis in the encephalitic tissue reaction (it is limited in the former chiefly to the vascular mesenchyma with very slight glia involvement, whereas in the latter gliosis is prominent), a clear-cut differential diagnosis is often impossible from an evaluation of individual preparations from isolated regions of the CNS. Thus, involvement of the spinal cord and brain stem is not by itself a criterion for differentiating the two diseases. This explains why in past decades such cases were misdiagnosed as poliomyelitis (sometimes with an atypical clinical course). They were not recognized because of the lack of supporting data.

The histopathological picture of ESME corresponds to that of a completely developed primary virus encephalitis of the disseminated type of "spotted polioencephalitides with meningeal involvement" [163]. It is characterized by discontinuous infection of widely separated parts of the CNS with distinct preference for the gray formations. It exhibits a striking constancy in attacking the spinal cord, brain stem, cerebellum, and mesencephalon (Fig. 3). Within this obligatory distribution pattern are only individual variations in intensity and extension of the encephalitic syndrome to the various grisea. However, the telencephalon must be regarded as an inconstant and facultative morphological characteristic of ESME and related arbovirus encephalitides [77, 163]. The most massive destruction of parenchyma occurs in the anterior horns of the cervicodorsal medulla, in the N. dentatus and cerebellar cortex, in the substantia nigra, and in the reticular brainstem and thalamic formations.

The gray substance of spinal cord shows the typical lesions with a predilection for the motor anterior horns, which cannot be clearly distinguished by histological means from those of poliomyelitis acuta anterior (Fig. 4a). In the brainstem, besides severe spotted infection of the tegmental nuclei there is constant involvement of the inferior olive and pons varolii gray substance, which occurs only rarely and to a very slight degree in poliomyelitis. Character-

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istic differences appear in the cerebellar affection. Whereas in poliomyelitis only the central and [illegible] regularly the vermis cortex have inflammatory lesions with destruction of parenchyma, the similar encephalitic lesions of the cerebellar cortex with loss and neuronophagia of the Purkinje cells (Fig. 4b), central nucleus, and medulla are to be regarded as a morphological feature of arbovirus encephalitis. The encephalitis process is, to be sure, often less intense in the brainstem ganglion, but it is more extensive than in poliomyelitis, where the severe lesions are confined to the deep gray substance, thalamus, and pallidum. In EEE and the brainstem nuclei are regularly involved with preference for the thalamus (Fig. 4c), putamen, and the N. caudatum less so; these are almost always spared in poliomyelitis. On the other hand, the complete sparing of the anterior hypothalamus (Nucl. paraventricularis and supraopticus) in WSME as compared with their preferred affection in poliomyelitis [77, 164] is noteworthy. Facultative telencephalon infection involves a diffuse dissemination of nodules over the entire cortex (without restriction to the motor central region typical of poliomyelitis), very severely affected claustrum, olfactory lobe gray substance, and subcortical medulla, which in poliomyelitis are generally free.

In protracted cases, spongy focal necrosis may occur in the cerebral cortex and medulla, brainstem ganglia, and cerebellar cortex (Fig. 4d. A similar phenomenon has been described in RSSE, JBE [165], EEE, WEE, and other arbovirus encephalitides. It is regarded as a facultative result of the inflammatory process due to severe injury to the perivascular glia [77].

The above histological findings show the possible extent of lesions in unfavorable cases, but they are useful only as an indication for a lesion pattern in the most benign forms of the disease, serous meningitis in particular. The clinician often finds no signs here of involvement of the CNS, and yet inflammatory changes should be present in the parenchyma [162], especially since it is reasonable to assume from animal experiments that the neurotropic virus first attacks the parenchyma, after which the meninges are successively damaged [85].

Every infection with WSME virus provokes in man the formation of specific antibodies, which are first detectable in the NT and HT and later in the CFR. The former are invariably found as early as the start of phase 2 [91], while the CF antibodies with good antigens cannot be detected until the 4th-7th days of phase 2 [90]. All these antibodies also appear after a clinically inapparent infection or infection without phase 2 (perhaps without phase 1 too). While the NT antibodies and hemagglutination-inhibiting antibodies presumably remain detectable for life in the serum, with the titer decreasing gradually, in the CFR less than a year after the infection there is a rapid decrease in the antibody titer, mostly of 4-8 units, but in the following years the titer drops very slowly in the CFR so that even

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after 4 years a residual titer can still be demonstrated. Although the titer level in the NT and HIT does not provide unconditional proof of a fresh infection, a titer of over 1:64 is usually found in the CFR; only titer values of up to 1:32 inclusive have been observed in the sera of persons unaware of having an infection of the CNS.



Fig. 3a: Brainstem ganglia: severe involvement of the thalamus with preference for the reticular nuclei, subthalamus, claustrum, and lenticular nucleus, with emphasis on the putamen. Affection of the basal olfactory lobe gray substance and sparing of the anterior hypothalamus. Occasional nodules of inflammation in the deep medullary formations.

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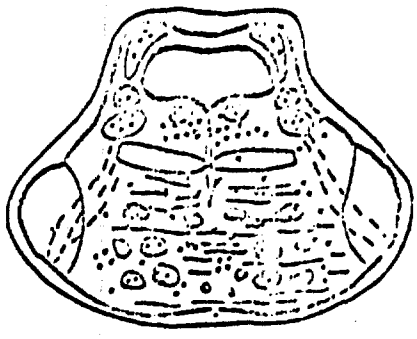


Fig. 3b: Pons: spotted confluent affection of the pons varolii. Severe involvement of the tegmental nuclei.

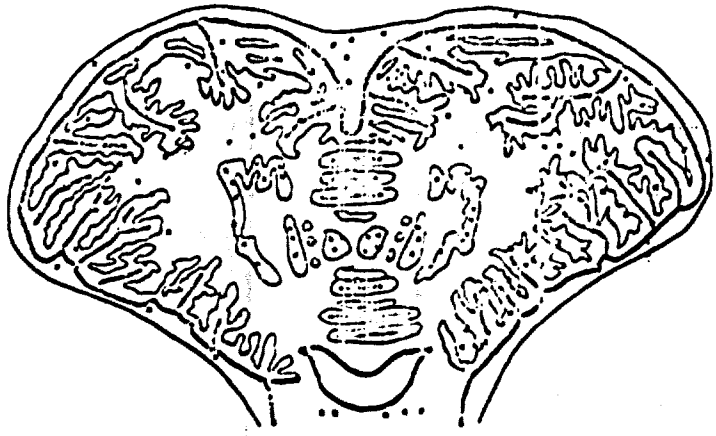


Fig. 3c: Cerebellum: diffuse dissemination of inflammatory lesions throughout the cortex. Nodular involvement of the central nucleus and medulla.

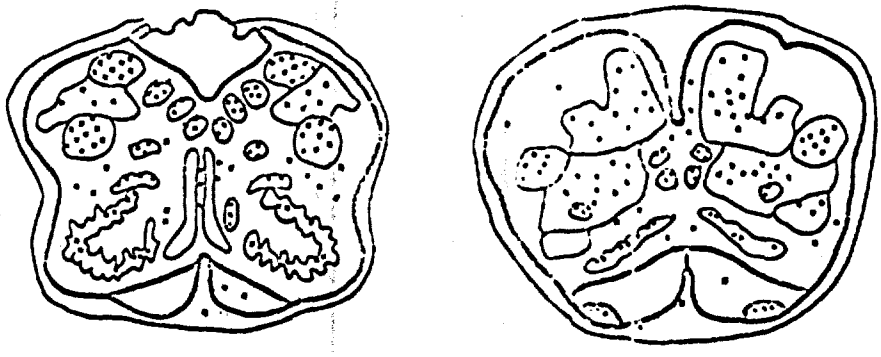


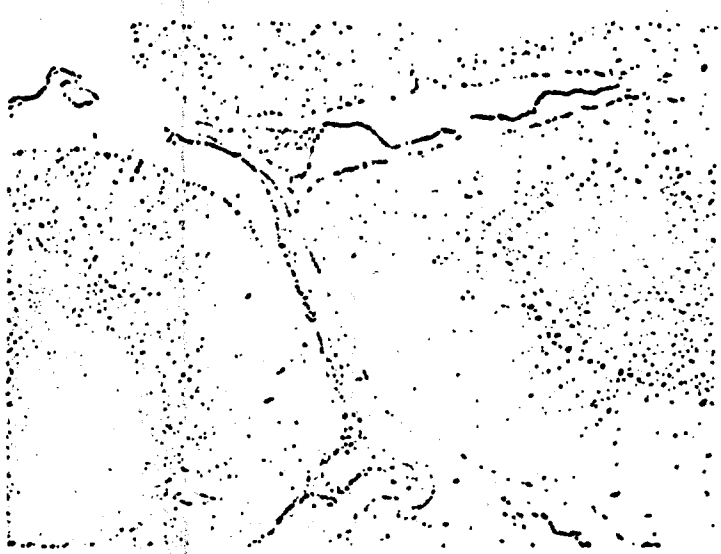
Fig. 3d: Oblongata: severe involvement of the reticular, motor, and sensory tegmental nuclei. Nodular involvement of the olives.

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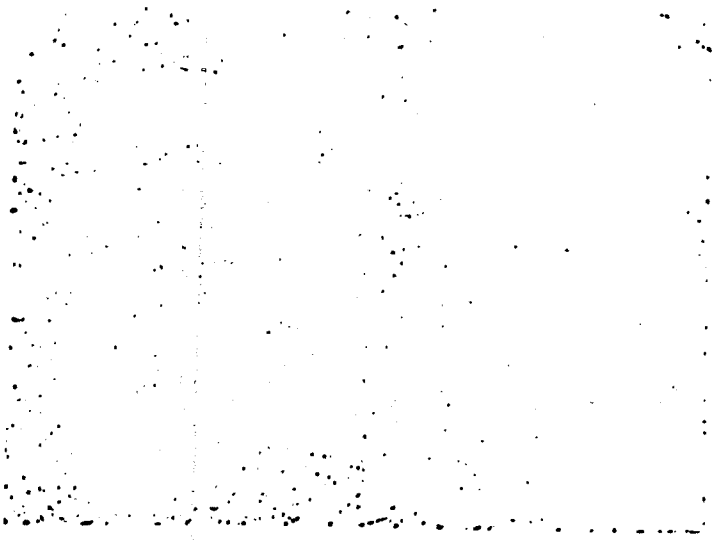
Fig. 4. Histology of the [illegible] [77].

(a) N. I. 75/56. Spinal cord, lumbar swelling. Spotty and nodular infiltration in the anterior horn with incomplete loss of the motor-root cells. Slight infiltration of tissue in the posterior horn. Fringe of round cells around the medial medullary canals. Mild diffuse meningitis. - Paraffin, cresyl violet, 12 X.

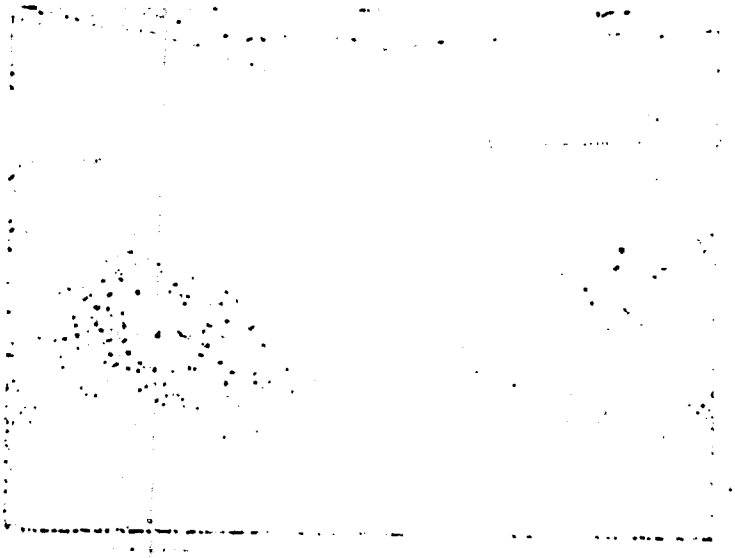


(b) N. I. 75/58. Cerebellar cortex. Inflammatory reactions in the meninges. Massive infiltrate pressing from the layer of Purkinje cells against the molecular layer. Partial loss of Purkinje cells. - Paraffin, cresyl violet, 40 X.

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(c) M. I. 119/61. Meninx cerebellaris. Numerous infiltrated nodules with lymphocytic vascular infiltrates with spotted nerve cell loss. Paraffin, cresyl violet, 45 X.



(d) M. I. 119/61. Cerebellar cortex. Spongy focal necroses. Almost complete loss of Purkinje cells. Residual infiltrates in meninges. Paraffin, cresyl violet, 90 X.

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On the basis of the [illegible] following; possibilities for serological investigation [illegible] are suggested:

First serum from [illegible] (serum dilution 1:5) or HI: if negative, rule [illegible]; if positive, suspect [illegible] (depending on the infection [illegible] investigations with all sera in the CF are necessary.

Second and all following [illegible] in the CF: indicative of TBEE are (a) conversion; (b) [illegible] (at least 4-fold) titer; (c) titer of 1:64, especially when no [illegible] can be detected owing to belated taking of blood or [illegible] blood sample is sent in.

[illegible]

TBEE occurs in Northern [illegible] especially in Sweden, on the Danish island of Bornholm and [illegible] Southern Finland, mainly on Aland Island (as Kumlinge disease). In Central Europe, the western boundary extends to about an imaginary [illegible] north-south line from the Baltic Sea to the Adriatic; east of [illegible] line the disease appears everywhere. In Southeastern Europe, it is known in Slovenia and in the northern part of Croatia, but it is not yet known just how far it extends to the southeast.

In West Germany, the [illegible] has been occasionally reported only on the Austrian border [illegible] in Oldenburg. It is endemic in the eastern portion of Lower Austria, Burgenland, Styria, and in Kärnten, but only a few cases have been observed in Upper Austria. Ixodes ricinus is very abundant in [illegible] these regions. A similar density of tick population is not known in other parts of Europe in which TBEE has not yet been observed. Small mammals, particularly wood and field mice, are presumed to be the virus reservoir; given a suitable density of vectors [illegible] vertebrates, the virus can circulate in a natural cycle between [illegible] and ticks and form a true focus [100, 140, 145]. Within such a focus [illegible] wild and domestic mammals as well as human [illegible] are implicated, but these are not of major significance in maintenance of the focus. Expression of this infection [illegible] in the fact that the virus can be isolated from the vertebrates or arthropods [illegible] specific sera. The human infection rate is 1% in Finland [136] and 1.2% (standardized on the basis of the 1961 census) in Lower Austria [67a]. The climatic conditions in Europe are paralleled by [illegible] accumulation of ticks in nature, which correlates well with the occurrence of human disease. Thus, Radda et al. found in systematically collecting ticks in a focus that the number of new patients lags by about 4 weeks (more or less corresponding to the incubation period, phase 1, and interval) behind the reported figures for ticks collected (Fig. 5).

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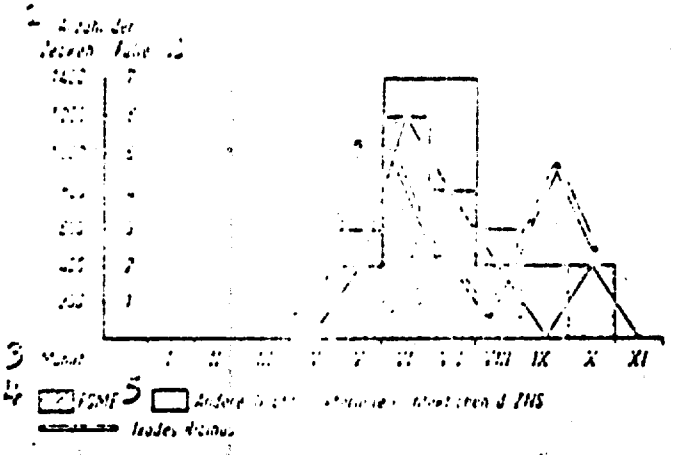


Fig. 5. Relationship between number of ticks collected and diseases of the CNS in [illegible] region, Neunkirchen (Lower Austria) in the years of 1962.

1 - Number of ticks
2 - Cases
3 - Month
4 - TBME
5 - Other (non-bacterial) infections of the CNS

In Central Europe there is a striking seasonal incidence of cases in May andJune with a maximum in July and a second but smaller peak in October. This is in contrast with the spread of other viruses, especially the enteroviruses (Fig. 6). In the North, however, the early July peak does not occur. The morbidity curve shifts in toto to the short warm summer with a maximum in August [180].

In view of the natural spread of this virus in the rural areas and the limited radius of action of the ticks, in general only those persons are exposed who go to this focus. Consequently, the average age of the patients is relatively high, i.e., 37.3 years (Table 1). Occupational exposure also plays a major role. For example, forest workers in an endemic region have three times the infection rate as compared with other inhabitants. Moreover, more males than females become sick (105:82). In questioning patients after a tick bite and consumption of raw milk, only about one-third of all the cases was attributable to the former. To be sure, this does not exclude a tick genesis, but it does point to other possibilities for infection. Drinking raw milk from viremic animals is not only a major source of infection in European Russia (two-wave milk fever), but must also be considered from time to time in Central Europe. After all, improper pasteurination of a mixture of cow's and goat's milk in Roznava (Slovakia) in 1951 resulted in an epidemic involving 360 persons [24]. Then there is the matter of contact infections, especially in slaughterhouses [1171], assumed to be a source of transmission of louping ill virus [206].

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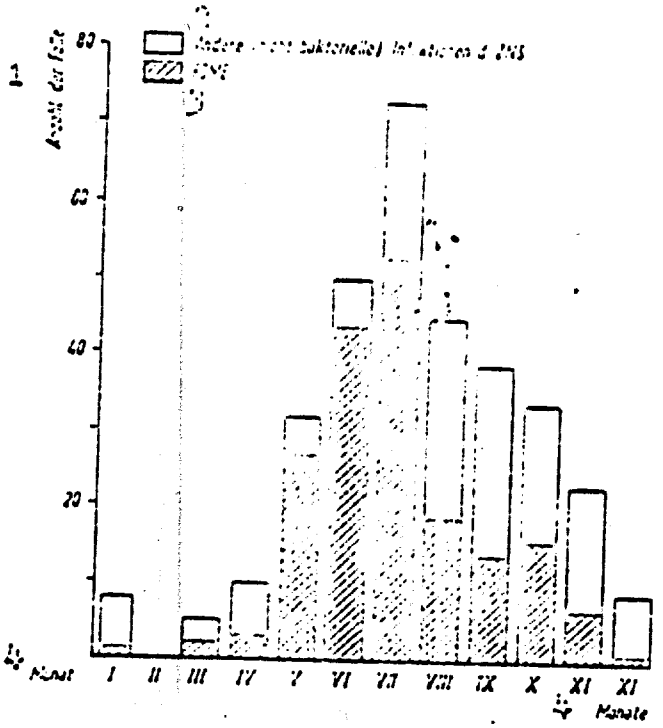


Fig. 6. Monthly distribution of CNS infections in an endemic ASMA region, Leonkirchen (Lower Austria), 1956-1962.

1 - Number of cases
2 - Other (non-bacterial) infections of the CNS
3 - ASMA
4 - Month

(4) Prophylaxis

With respect to prophylaxis, a distinction must be made between individual prophylaxis and sanitary measures in a focus. Individual prophylaxis is related to the passive and active immunization of human beings. Passive immunization can in practice be undertaken only in laboratory accidents when an infection is established and first aid is given in the form of administration of hyperimmune globulins. The important thing here is timeliness, for a manifest disease of the CNS (phase 2) cannot be successfully treated.

For active immunization, efforts are being made to produce vaccines with viruses inactivated of mice (by formaldehyde- and heat-treated ...) from tissue cultures [23, 44, 71]. Like the vaccines used in Russia [99], because of the complications that arise from the use of vaccines prepared from the brains of mice suckling mice [73]. In the future it may be possible to use attenuated live vaccines. Promising results

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have been achieved in field [illegible] (cf. Loudan; 111) and in laboratory experiments [illegible] both with the Langat TP-21 strain alone and in combination with yellow fever-17-D vaccine and an attenuated West Nile strain. [illegible] later vaccination should be considered in Central Europe for laboratory workers and especially exposed persons like [illegible] forest workers.

General sanitary measures [illegible] directed against the vector (ticks), its reservoir [illegible], or possible virus eliminators (large animals). Effective tick control in Central Europe has been described only by Sinnecker [178].

Eradication of rodents on a small experimental tract still appears to be unrealistic. On the other hand, it is not difficult to block alimentary infection of human beings either by "high" pasteurization of milk in the usual way at 85° or by actively immunizing the large animals because immunized animals no longer eliminate the virus with their milk [92].

5. Hemorrhagic Fevers (HF)

By HF is meant an acute [illegible] arbovirus infection with hemorrhagic diathesis accompanied by a characteristic damage to the capillaries in various organs. This hemorrhagic diathesis represents a peculiar phenomenon of an arbovirosis which can occur by itself associated only with fever or as an aggravating complication following another arbovirus infection as, e.g., in yellow fever, EEE, etc.

Such disease patterns have been observed in different parts of the world for years. Hence, these diseases are generally named after their geographic origin. Whether all the hitherto described (etiological but not identified) HF relate to a single arbovirus infection is very doubtful. Moreover, despite intensive efforts, arboviruses as the causative agent have been isolated only in a few places (cf. table).

(a) Kyasanur Forest Disease (KFD)

In the spring of 1957, many monkeys died in Kyasanur Forest in Shimoga District (Mysore), an event that was related to sicknesses of persons who had worked in this wooded region. Systematic studies led to isolation of the causative agent (KFD virus) from the organs of dead monkeys, from the blood of acutely affected persons, and from ticks of different genera, Haemaphysalis spinigera in particular [210]. The virus belongs to group B and is a member of the TBE complex. It is different, however, from the virus of Siberian HF (Omsk type) in antigen structure [96]. Monkeys (Macaca radiata and Presbytis entellus) are the natural reservoir. The infection spreads during the rain-free months from January to June simultaneously with the seasonal development of the ticks. The monkeys become sick and also have the clinical symptoms of a hemorrhagic diathesis, which may be fatal. Until now this infection has been observed only in this one place in India.

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In man the disease sets in acutely after an incubation period of 5-6 days. High fever, headache, albuminuria, and severe leukopenia and thrombocytopenia, which cause hemorrhages in the mucous m. organs in addition to direct injury to the vascular walls, may last 9-10 days. The virus can be regularly isolated from the blood during this first febrile phase (Fig. 7 [78]). The hematological indices slowly return to normal when the fever subsides. A second febrile phase may occur after a fever-free period of 1-3 weeks. It has the same symptoms as the first phase (Fig. 7), but sometimes follows the course of a meningo-encephalitis. Antibodies can be detected in the NT following the viremic stage in the second week of the sickness and in the CFR. The fatality rate is 10%.

Hemorrhagic Fevers

1 Geograph. Verbreitung	2 Klinische Bezeichnung	3 Ätiologie	Vektor
4 Nord-Skandinavien *[54]*	Nephropathia epidemica	12 unbekannt	12 unbekannt
5 Ungarn *[128]*, Jugoslawien, Bulgarien *[4]*, Transkarpathien (Bukowinien) *[5, 54]*	Hämorrhagische Nephroso-Nephritis 8 Bukowinisches Hämorrhagisches Fieber	12 unbekannt	Acarina (?)
6 Krim *[54]*, Astrachan	9 Hämorrhagisches Krimfieber	12 unbekannt	Acarina (?)
Uzbekistan *[54, 55]* (Zentralsibirien)	Uzbekistan Hämorrhagisches Fieber	12 unbekannt	Acarina (?)
Baraba-Steppe	Omsker Hämorrhagisches Fieber	TBE-Virus Typ Omsk	Ixodidae
Indien (Distrikt Shimoga)	Kyasanur Forest Disease	TBE-Virus Typ KFD	Ixodidae
Malaya, Thailand, Philippinen	Singapur-thailändisches-philippinisches Hämorrhagisches Fieber	Dengue-Virus Typ 2, 3, 4 Chikungunya-Virus	Culicidae
7 Fern-Ost-Sibirien *[54, 55]*, Mandschurei, Korea	10 Fern-Östliche Hämorrhagische Nephroso-Nephritis	12 unbekannt	Acarina (?)
Argentinien	Argentinisches Hämorrhagisches Fieber	Junin-Virus	Acarina
Bolivien	Bolivianisches Hämorrhagische Fieber	13 verwandt mit Junin-Virus	12 unbekannt
USA *[112, 166]*	11 Hämorrhagisches Fieber von Odocoileus virginianus (Epizootie)	Epizootic Hemorrhagic Disease (EHD) virus	12 unbekannt

1 - geographic distribution; 2 - clinical name; 3 - etiology; 4 - Northern Scandinavia; 5 - Hungary, Yugoslavia, Bulgaria, Transcarpathia (Bucovina); 6 - Crimea; 7 - Far East-Siberia; 8 - Bucovina hemorrhagic fever; 9 - Crimean hemorrhagic fever; 10 - Far Eastern hemorrhagic nephrosonephritis; 11 - hemorrhagic fever from Odocoileus virginianus (epizootic); 12 - unknown; 13 - related to Junin virus

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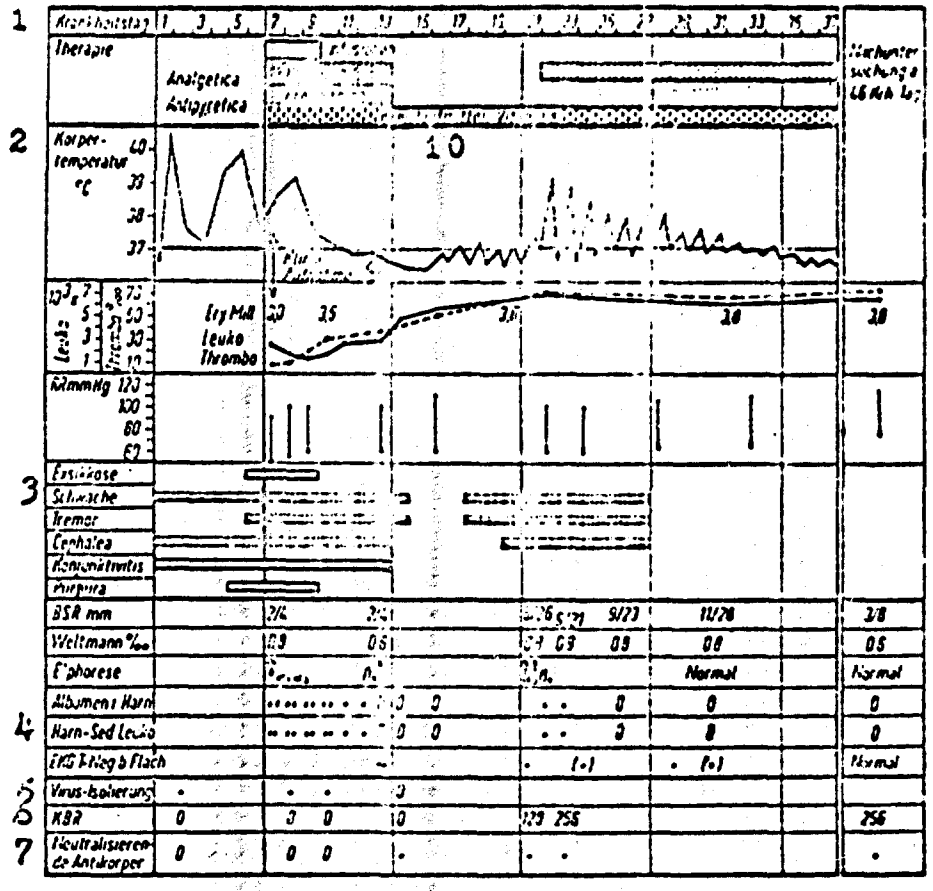


Fig. 7. Laboratory infection with KFD virus

1 - day of sickness; 2 - body temperature; 3 - weakness; 4 - urine; 5 - isolation of virus; 6 - CFR; 7 - neutralizing antibodies; 8 - follow-up examination on 40th day of sickness; 8 - admitted to clinic; 10 - circulatory agent, vitamins

(b) Omsk Hemorrhagic Fever

A disease of man with a hemorrhagic diathesis has been observed in the Baraba Steppe in the Omsk region (West Siberia) since 1944. The causative agent, isolated in 1947 by Shumakov, belongs, like KFD virus, to the TBE complex and is transmitted by Dermacentor pictus and Dermacentor marginatus. The host reservoir of the virus is still unknown [54]. The former appears mainly in the northern part of the forest-steppe, the latter in the southern part. Neither is found, in general, with Ixodes persulcatus so that Omsk HF and ESME are not observed near each other [12].

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(c) Thai-[illegible] Hemorrhagic Fever

Epidemic outbreaks of HF have been observed in Southeast Asia since 1954. Those in the severe 1956 epidemic in Manila with over 1200 patients led to the isolation of dengue virus types 3 and 4; those in 1958 in Bangkok, dengue virus types 2 and 4 and Chikungunya virus [182].

The surprising thing about this HF is that it only struck children who became sick with a fever and hemorrhagic diathesis and circulatory collapse, and in Bangkok with hepatomegaly as well. The case-fatality rate was about 10%. The causative agents were probably transmitted chiefly by Aedes aegypti. The epidemics broke out in Manila in April and May (before the rainy season) and in Bangkok from July to October (during the rainy season).

A peculiarity of the disease is that a relative uniform and characteristic set of symptoms, i.e., the HF, is caused by different arboviruses even from different groups (A and B), while in another place the dengue type 2 and Chikungunya viruses can give rise to a typical dengue fever (cf. dengue fever below).

(d) Argentine Hemorrhagic Fever

In 1958 Greenway et al. [61] were the first to isolate virus from the organs of patients with HF. It was observed for several years northwest of Buenos Aires and is also called "Mal de los Rastrojos". This "Junin virus" was later isolated from rodents. Contrary to the original research results, the virus does not belong to the TBE complex but forms a group with the Tacaribe virus (Tacaribe group). The virus is presumably transmitted by mites. Whether the vectors are actually mites from the Gamasina group cannot be decided for the time being from isolation of the virus from Echinolaelaps echidninus. The incidence of the disease in man reached a peak in May-June (winter!), especially among farmers.

The disease increases in severity with age. Hemorrhages in the kidneys and brain may be fatal.

6. Dengue Fever

Dengue is a febrile disease of man caused by virus and transmitted by mosquitoes. It is characterized by pain in various parts of the body, especially the joints, exanthema, and lymphadenopathy. The clinical picture of the disease has been known for several centuries in the Far East. Bancroft (1[illegible]), was the first to discover that it is transmitted by Aedes aegypti. Craig and Ashburn (1907) identified the causative agent as a virus. Isolation and cultivation of the virus in mice and well as the discovery of the variety of antigen types were achieved only after protracted investigations during World War II [151].

Today a distinction must be made between the clinical concept "dengue fever" and the microbiological term "dengue complex". The former is a definite human disease that may be caused by different arboviruses, whereas the latter comprises 4 antigenically related viruses of group B called dengue virus types 1-4. Types 1 and 2 are largely responsible for the clinical symptoms of "dengue fever", while types 3 and 4 (frequently type 3 alone) cause a "hemorrhagic fever" (q.v.) in children.

The dengue virus is 17-25 μ in size. It can be concentrated in suckling mice and in tissue culture. The individual types can be differentiated by means of immune sera in the CFR and NT.

Extraneural and intracerebral injection of a fresh strain results only in clinically inapparent (although histologically and serologically manifest) infections in monkeys. However, poliomyelitis-like lesions can be provoked in monkeys (chimpanzees) with mouse-adapted strains [149].

The diagnosis of dengue in cases with characteristic clinical symptoms (at least in the second fever phase). At the beginning of the illness (first fever phase) the virus circulates in the blood and can be isolated by inoculation of suckling mice. During convalescence antibodies can be demonstrated in the NT and HIT as a reflection of the homologous virus strain immunity.

The disease in man sets in after an incubation period of 5-8 days with fever, headache, and pain in the muscles, sacral region, and joints (rheumatic type). The initially high fever (40° C) drops on the 3rd-4th day of the sickness but rises to 40° again on the 5th day. It subsides on the 7th day.

The fever and characteristic pains are associated with an exanthema appearing mostly between the 3rd and 5th days and disappearing rapidly. The tendency to hemorrhagic diathesis (as with types 3 and 4) is very slight in a typical dengue fever, although petechiae in the exanthema and in case of death (rare) hemorrhages in the region of the serous membrane and mucosa have been described.

The peculiar syndrome is responsible for the name "dengue", which comes from the Spanish "dengoso" or "denguero" meaning "affected, coy, prudish" because the unusual body position enforced on the patients by pain results in their walking with legs wide apart (English "dandy fever").

The dengue viruses types 1 and 2 are, like yellow fever virus, transmitted chiefly by Aedes aegypti. Man seems to be the only reservoir, especially since thus far, except in experimental infections of monkeys, no other natural host has been found.

This man-mosquito-man cycle can [illegible] be maintained in tropical and subtropical regions (without winter), if the human and mosquito populations are sufficiently dense. However, it does not exclude the existence of another basic cycle with the participation of a wild vertebrate (monkey?)

The range of both dengue virus types corresponds to the tropical and subtropical zones of the Middle and Far East, Africa, and America. These viruses are also transmitted by [illegible] culicid species. In Europe an epidemic broke out in Greece in 1927-1928 and attacked more than a million persons. Type 1 was retrospectively determined as the causative agent [191].

Prophylactic measures are aimed primarily at controlling the mosquitoes. The rigorous measures taken against anopheles by Europeans in their colonies enabled them, unlike the natives, to escape o'nyong-nyong fever. Vaccine with attenuated dengue 1 and 2 strains was used.

Chikungunya virus (Africa subtype) (group A) was isolated for the first time in 1952 in East Africa from the blood of patients and mosquitoes. At that time there was an epidemic of a benign dengue-type fever among the natives, mostly with the characteristic joint pain and diphasic fever with an exanthema. The absence of adenitis distinguishes chikungunya fever from dengue.

The virus is transmitted by numerous culicid species. The main range of distribution is South and East Africa and the Congo, although it has also been isolated (Thailand subtype) in Thailand from the blood of children with HF (cf. HF).

O'nyong-nyong virus (group A) is closely related to chikungunya virus and was first isolated from the blood of patients in Uganda in 1959. An epidemic of a benign dengue-type fever was then raging among the natives of Uganda, Kenya, the Congo, and the Sudan, with more than 750,000 persons affected. The clinical picture is very similar to that of dengue, but is called o'nyong-nyong by the Africans. The virus is spread by Anopheles, from which it can be regularly isolated. A natural host reservoir has not been discovered yet [43, 69, 168, 208].

7. Yellow Fever

The original homeland of yellow fever cannot be precisely determined, but it is now believed that the virus was imported to the West Indies from Africa in the 17th century. In the 18th and 19th centuries severe yellow fever epidemics occurred in Central and South America. In 1881 Findlay assumed a relationship between mosquitoes and the distribution and transmission of the virus to man. Confirmation was provided by the studies of the American yellow fever commission in Cuba headed by Reed (1900/1901). They were able to show that the

causative agent passes through a bacterial filter and circulates in human peripheral blood during the first three days of fever. It is acquired by blood-sucking [illegible], which can transmit the virus further after about 12 days. These findings were given practical application and it seemed as though mosquito control on the American continent could lead to eradication of yellow fever in the cities until a wider cycle of the virus in nature was discovered in the course of the 1928 epidemic in Rio de Janeiro and in experimental research [185]. Finally, Theiler [189] succeeded in infecting mice intracerebrally and culturing the virus. This led to the development of vaccine from attenuated virus strains [179].

Electron-optical studies have shown the yellow fever virus (Asibi strain) to be 25-27 mμ in size [16]. It survives in 50% glycerin solution and in a lyophilized state for a long time. Freshly isolated strains have viscerotropic and neurotropic characteristics, the former being dominant in the natural cycle of the virus. A human being or experimentally infected monkey develops a viremia and hematogenic involvement of the liver in a few days. Encephalitis does not follow intracerebral injection of this strain. It may be that a hyperimmune serum is produced at the same time which neutralizes only the viscerotropic elements.

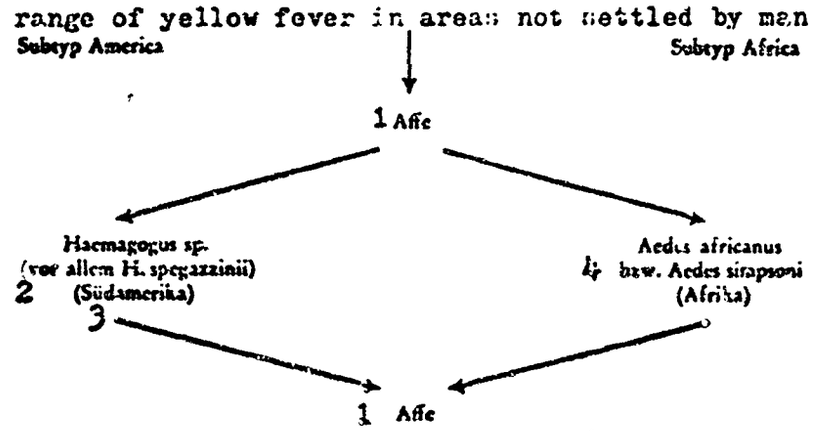
Theiler was able to adapt the virus to mouse brain and to select the neurotropic elements so that such strains could produce encephalitis in mice and monkeys after a short incubation period. However, after subcutaneous injection 5-10% of the monkeys died of encephalitis. Yet the virus had lost its viscerotropic properties as a result of the mouse brain passages so that it was found in the CNS and various glands of the monkeys that died of encephalitis but not in their blood or liver.

Haagen and Theiler [68] described multiplication by the neurotropic components of the virus in Maitland cultures, but Lloyd et al. [98] were the first to grow in tissue culture the Asibi strain which possessed both neurotropic and viscerotropic characteristics. This led in the course of the passages to partial weakening of the viscerotropic components in this originally pantropic virus strain without strengthening of the neurotropic characteristics. A branch line of these passages was called 17D and is now used as an attenuated vaccinal strain. After extraneural injection this strain causes only a slight viremia in monkeys and is not followed by encephalitis or hepatitis. However, after intracerebral injection encephalitis develops regularly in mice and in about one-third of experimental monkeys. At present this strain is cultivated for the preparation of vaccines in fertilized chicken eggs. In addition, laboratory strains of yellow fever are also grown in different tissue cultures under conditions of plaque formation. The American and African subtypes can be distinguished serologically [41a].

The initial clinical symptoms of yellow fever appear after an incubation period of 3-6 days. The initial febrile phase (=viremia) lasts 3-4 days and after a brief remission of 1-2 days fever again occurs as a reflection of organ involvement. The course varies from case to case - from clinically inapparent or only with mild fever and headache to moderately severe cases with fever, jaundice, and albuminuria, and to severe cases with complications resulting from protracted hemorrhages. While the first phase of the viremia sets in acutely with fever, leukopenia, and the usual characteristic but nonspecific attendant phenomena, the second phase shows the typical signs of liver and kidney damage. In the liver, especially in the intermediate zone of the lobes, can be seen necrosis and fatty degeneration, whereas the cells on the periphery and in the center are relatively well preserved. The necrotic cells acquire a hyaline appearance and are called"councilman bodies."The capillaries are very dilated, but there are no signs of injury to the Kupffer cells or bile duct. The resultant icterus appears during phase 2; an early appearance implies an unfavorable prognosis. The kidneys too show no signs of an inflammatory reaction but fatty degeneration of the tubuli. Albuminuria approximately matches the severity of the icterus. Oliguria may turn into a prognostically unfavorable anuria. A decrease in quantity of urine, diminution of albuminuria, and excretion of bile pigment, on the other hand, are prognostically favorable signs.

The hemorrhagic diathesis is probably a sign of direct injury to the vascular wall (cf. HF), with a decrease in vitamin K synthesis resulting from the liver damage. A tendency to bleeding is everywhere (skin, viscera); hemoptysis is particularly to be feared. Circulatory impairment is manifested in a low pulse rate (with elevated body temperature). At the beginning of the first phase the blood picture is characterized by leukopenia; later the blood coagulation time is lengthened. Death may occur around the 6th or 7th day as a consequence of renal insufficiency and hepatic coma. Otherwise the patient recovers without developing chronic kidney and liver damage and acquires immunity.

The range of yellow fever in areas not nettled by man follows the cycle:



1 - monkey; 2 - chiefly; 3 - South America; 4 - or

This cycle maintains the virus under natural conditions and because of its primitivity is to be regarded as the main cycle of yellow fever. This form is also called "sylvan" or "jungle yellow fever". In such a cycle man is only occasionally infected, that is, when he chances to enter such a focus. Only individual persons are involved so that it is a matter of a sporadic occurrence or perhaps also an occupational disease. Should, however, a person infected in a focus return to a settled area (during the viremia) in which Aedes aegypti is present, there is the possibility of a transformation into an "urban yellow fever", the following cycle ensuing:

Man → Aedes aegypti (vector) → Man (reservoir) →

Whether this imported yellow fever gives rise to an epidemic outbreak or it remains a sporadic case depends on the degree of immunity (perhaps also with other strains of group B) of the population.

Aedes aegypti is a mosquito that lives in close association with man. It prefers to lay its eggs in bodies of water, perhaps also in tree and bamboo holes, mostly on top of the water. The eggs are highly resistant to dryness and very little water is needed for the larvae to hatch. The females bite mostly in the early hours of the morning, almost always in a closed space. 22-25° C is the temperature needed for their development and activity. Hence, they may appear in any warm region of earth, including South Europe. The external incubation period is 4 days at 37°, 8-10 days at 25°, and 30 days at 18°. The life-span of the female is relatively brief (particularly since she does not overwinter), ranging from 2-5 weeks.

Experimental studies have shown that many mosquitoes besides Aedes aegypti can be infected with the virus. This is consistent with reports on the isolation of the yellow fever virus from various culicids in America and Africa. How far they can be held responsible for a spreading of yellow fever depends, on one hand, on the density and behavior (choice of hatching place, etc.) and, on the other hand, on contact with human beings so that an endemic or epidemic can result.

The following are regarded as yellow fever endemic zones, according to the latest information (1962) on protective inoculations in international travel: Africa (with the exception of a few major cities) on both sides of the equator to the 15th north and south latitudes, South America (with the exception of the major port cities and Panama Canal zone) north of the equator and in the interior to about 15° S. Lat.

Laboratory diagnosis of yellow fever is based on:

(1) Isolation of virus from blood (phase 1) and liver (viscerotomy or autopsy);

(2) Presence of antibodies (conversion or rising titer during phase 2);

(3) Histological examination of liver punctates.

Preventive measures include control of mosquitoes and individual prophylaxis by vaccination. Control measures are aimed mainly at eradicating Aedes aegypti since this species is largely responsible for epidemics. Such attempts were started at the turn of the century and by 1925 caused a considerable reduction in the incidence of the disease in South American port cities. During and after (since 1947) World War II these campaigns were strengthened by the use of insecticides. By 1960 in numerous South American countries Aedes aegypti had become an insignificant member of the total biocenosis and no longer an acute danger to man.

The American vaccine with the 17D strain and the French vaccine with the Dakar strain are now available for individual prophylaxis. The 17D strain is concentrated in chicken embryo and subcutaneously inoculated, while the Dakar strain is grown in mouse brain passages, after which the brains are dried and suspended in gum arabic for use. The inoculation is carried out after scarification. The advantage of the American vaccine is that it is well tolerated, whereas neurological complications frequently follow the use of the French vaccine. On the other hand, the French vaccine is suitable for mass inoculations because of the simple technique required.

Contrary to all recommendations not to couple yellow fever inoculation with other live vaccines, reports have recently come in on success achieved with simultaneous inoculation (small pox + yellow fever) in Nigeria [111]. The indication for this simultaneous inoculation was based on the need to carry out both inoculations on a large scale in view of the particular local conditions.

According to international determinations, a rather reliable immunobiological protection is afforded for 6 years by a yellow fever vaccination authorized by the World Health Organization.

8. Meningoencephalitides Whose Causative Agent Is Transmitted by Mosquitoes

Some arboviruses transmitted by mosquitoes are capable of causing meningoencephalitis in man and occasionally, under natural conditions, in animals. From the virological standpoint, these neurotropic viruses offer no unusual features. In general, they can be concentrated after extraneural administration in adult mouse brain, although tissue culture is now preferred in normal practice.

The clinical picture presents with considerable variations all the characteristic but nonspecific symptoms of a virus infection of the CNS, as in TBE, so that the diagnosis must be based in each individual case only on systematic virological-serological examinations (isolation of virus from parenchyma, often from fluid as well). Involvement of the CNS is invariably preceded by a clinically uncharacteristic initial phase of viremia (isolation of virus from the blood is theoretically possible. Since neutralizing antibodies are almost always to be expected in the patient's serum at the beginning of phase 2 (involvement of the CNS), in routine diagnosis the CFR is preferred to the HIT or NT.

In contrast to the relatively uniform picture of human disease, the individual viruses responsible differ from one another in antigen structure, vector, host reservoir, and geographical distribution.

(a) American Equine Encephalitides

Epizootics among Equidae with involvement of the CNS have been reported since the end of the 19th century, especially in the western part of the United States. Meyer et al. (1931) were the first to isolate the WEE virus in California from the brains of dead animals; Ten Broeck and Merrill and Gietner and Strahan (1939) isolated the EEE virus on the east coast of the United States; Beck and Wyckoff (1938) isolated the VEE virus in Venezuela. Along with these isolations the investigators also discovered the causal connection between human meningoencephalitides and this virus in the West and Midwest (WEE) and east coast of the United States (EEE).

These three viruses belong to group A and differ from the other arboviruses in their unusual pathogenicity for Equidae even after peripheral infection (mosquito bite!). Electron-optical studies showed the WEE "provirus" to be 22 mμ in size. The mature WEE virus (like the VEE virus) [121] consists of a thick nucleus 30 mμ in size and a peripheral membrane with a diameter of 45-48 mμ [115].

Since the viruses can be inactivated with formalin, vaccines can be used to protect not only horses but also exposed laboratory workers against all three encephalitis viruses.

(i) Western Equine Encephalitis (WEE)

The virus is widespread in the United States, like SLE virus, only west of the Mississippi. It is also found in Canada, Brazil, Uruguay, and Argentina. The disease in human beings appears between June and September, with the peak in July, and it frequently attacks children. Besides sporadic cases, there are frequent reports of epidemics among horses and human beings. The fatality rate is between 7-20% (average, 10%). The virus is transmitted chiefly by Culex tarsalis. Neither the natural virus reservoir nor the places

of overwintering are known. There is no doubt that migratory birds as well as domestic fowl play a special role, the former possibly being responsible for importing the virus into the focus afresh every year. The virus can hibernate in experimentally infected water snakes (Thamnophis sp.) and in Culex tarsalis imagines [144, 192].

Besides the original natural hosts, all the wild and domestic animals as well as human beings living in a focus can be infected by female mosquitoes, but only Equidae and man develop a meningoencephalitis. These, however, are of less significance in maintaining the cycle of the virus in nature.

(ii) Eastern Equine Encephalitis (EEE)

The North American subtype of the virus appears in Eastern Canada and the United States, in Mexico, and in the West Indies. The Central-South American subtype appears in South America (Panama, Brazil, Argentina) and Southeast Asia. The disease strikes human beings, children and teenagers in particular, in the late summer and early fall. There are also sporadic minor epidemics.

This virus is highly pathogenic for man due to the hemorrhagic diathesis. Phase 1 is quite pronounced (fever, vertigo, vomiting, headache), the hemorrhagic meningoencephalitis developing in phase 2. The mechanism of action on the vascular system is still obscure. The fatality rate is high, amounting to 74% of the human beings and 90% of the horses infected during the first recognized epidemic (1938) in Massachusetts. These high death rates are to be regarded as a reflection of a high fatality rather than high mortality rate because of the persons with NT antibodies who did not suffer from a disease of the CNS.

Little is known with certainty about the natural vector (main vector for maintenance of the cycle in nature. To be sure, the virus has been isolated from Culiseta, Mansonia, Culex, Anopheles, occasionally from Culicoides, various Simuliidae, and even Acarina under natural conditions, with Culiseta melanura assumed to be responsible for maintenance of the main cycle (wild birds — Culiseta — wild birds). However, antibodies against EEE virus have been found in numerous vertebrates so that definite conclusions cannot be drawn as yet. In addition, the overwintering of the virus, as in WEE, is still unsolved. Just as in WEE, horses are not responsible for maintenance of the cycle and, like man, they are to be regarded rather as a susceptible terminal member of the infection chain.

(iii) Venezuelan Equine Encephalitis (VEE)

The virus is widespread in equatorial South America. Unlike WEE and EEE, the VEE virus causes encephalitis in Equidae, but not in man. It is a long-phase febrile disease, frequently dengue-like

in character (pain in the joints and limbs). Another peculiarity is that the virus is present in the nasopharyngeal space of infected persons and horses so that transmission without a vector, in contrast with all the other arboviroses, cannot be ruled out. The virus is also excreted by infected horses with urine and by experimentally infected mice with feces. This surprising excretion and secretion of the virus is undoubtedly responsible for the frequency of laboratory infections.

Under natural conditions, the VEE virus, as shown by the isolations from many species of Culicidae, is transmitted to human beings and horses by Mansonia titillans and Aedes taeniorhynchus. It is still not known whether any species as chief vector plays a special role in maintenance of the virus in nature. Wild birds are conjectured to be the virus reservoir, in which case Aedes trisenitatus would function as the vector. Unlike WEE and EEE, the VEE virus is found only in the tropical zone with a constant climate so that there is no problem here of overwintering.

(b) St. Louis Encephalitis

Meningoencephalitis epidemics broke out in the summers of 1932 and 1933 in the midwest of the United States; the causative agent was isolated in 1933. The virus belongs to group B and is related in antigen structure to JBE, MVE, WN, and Ilheus viruses. Today the SLE virus' range is from the Pacific Coast of the United States to the Midwest (like WEE virus), Panama, West Indies and Ecuador, although the location of epidemics changes from year to year. The most important and best studied epidemic occurred in Houston, Texas in 1964.

The incidence of the disease is highest in the late summer and early fall (WEE, about a month later), the peak occurring in August-September, with different age groups preferentially attacked. The clinical symptoms are often inapparent (estimated age rate inapparent = apparent = 64 - 209 :1 [25] or so mild that for want of inclusion of all cases an exact fatality rate cannot be determined (but it is surely low).

The virus is isolated chiefly from Culex tarsalis, also from numerous other culicids. The natural virus reservoir is migratory fowl. Poultry, domestic animals, and wild mammals are also infected by mosquitoes. Overwintering of the virus is still unknown.

(c) California Encephalitis

California encephalitis virus (CEV) was isolated for the first time from Culex tarsalis in California [69a, 69b, 144a] and from a hare [29]. In addition, antibodies against CEV were found in three patients with encephalitis in California. The significance of this virus in connection with an infection of the human CNS is still unclear.

(d) Japanese B Encephalitis (JBE)

The first descriptions of this disease date back to Kawakita (1871), but epidemics have been regularly recorded in Japan only since 1924. In 1934 Hayashi was the first to isolate the causative agent by transmission to monkeys. In 1935 this infection was named B-encephalitis to distinguish it from von Economo (A) encephalitis. The virus belongs to group B and is serologically related to SLE virus. Its range now extends to East Asia, India, and Micronesia. The incidence is highest in the temperate zones from mid-August to mid-October (autumn encephalitis) [176], with children and teenagers preferentially attacked in endemic regions. The clinical symptoms vary from unapparent to fatal diphasic forms (as in TBE) with a fatality rate of about 8%. Encephalitic forms appear to be more common than paralytic forms.

The virus is transmitted by various culicids, especially Culex tritaeniorhynchus. Various wild fowl appear to be the natural virus reservoir, although swine and horses are also infected; the latter may be responsible for a subcycle. Inoculation with formolized viruses from man and horses helped to reduce morbidity considerably in Central and South Japan [165].

(e) Murray Valley Encephalitis (MVE)

An encephalitis epidemic broke out in Australia for the first time in 1917-1918 (70% fatality rate), which in a milder form in the following years was also presumably caused by the same agent (Australian X disease). Another epidemic broke out in Eastern Australia in 1951 and the causative agent was isolated. The agent isolated in 1917 disappeared meanwhile, but serological examinations demonstrated the identity (or close relationship) of the two viruses.

MVE virus belongs to group B and is closely related to JBE virus. The virus is widespread in Australia and New Guinea. It has been isolated from various culicids, especially Culex annulirostris, undoubtedly the major factor in nature.

9. Pappataci Fever (Phlebotomus Fever)

The clinical pattern of pappataci fever has long been known in the Adriatic and Mediterranean area. The disease was first described as a clinical entity by Pick (1886). As early as 1909 Doerr, Franz, and Russig showed that the causative agent is a virus that circulates in the patient's blood and is transmitted by Phlebotomus papatasi. The first demonstration of the virus followed yellow fever studies in Cuba and later a commission of army doctors discovered an arbovirus cycle in Europe [75]. During World War II Sabin [150] succeeded in isolating several strains in the course of comprehensive investigations on allied soldiers in the Mediterranean area and in distinguishing

two serologically different types on the basis of experiments on volunteers - Sicilian type (1943) and Neapolitan type (1944).

The virus is about 50 mμ in size; it was isolated from phlebotomi and patients' blood through transmission to suckling mice. It can also be adapted to adult mice or tissue cultures. Otherwise it is transmissible only to human beings. It appears in human blood 24 hours before and after the beginning of the clinical symptoms. Onset of the disease after a brief incubation period (3-6 days) is acute with a high fever, chills, headache, and sensitivity to light. Since the face and conjunctiva are reddened, the disease used to be called (in the 19th century) "dog's disease" (red eyes!). The fever subsides after 2 or 3 days, becoming normal on the 4th day. The leukopenia and bradycardia are striking. However, enlargement of the spleen and exanthema do not occur. Differential diagnosis is a problem owing to the acute beginning - malaria (blood picture) and dengue (exanthema). The disease produces a type-specific immunity, which can be definitely demonstrated in the NT (better than in the HIT and CFR).

Under natural conditions the virus is transmitted by Phlebotomus papatasi. The Phlebotominae constitute a subfamily of the Psychodidae and, consequently, are closely related to the Culicidae. They are blood-sucking (humans and domestic animals) ectoparasites and can easily be distinguished from mosquitoes by their small size (only about one-third that of the Culicidae) and sandy yellow color (sand-flies). Like the many other familiar phlebotomus species, Phlebotomus papatasi prefers to stay in the living and sleeping quarters of man. The range of flight, unlike that of most Culicidae, is short and limited to 100-200 meters around the hatching places. The latter (in contrast with those of mosquitoes) are found in heaps of moist, organic materials as in rodent burrows, cracks in walls ("ruin disease"), garbage, dung, etc. A complete developmental cycle takes about six weeks so that there are usually two generations a summer. The imagines (female), like mosquitoes, feed on blood. Their bites (often several for a meal) are particularly painful.

Man serves as the principal virus reservoir when bitten during viremia (about 2 days) by imagines. The extrinsic incubation period in imagines fluctuates between 6 and 10 days. In addition, it is maintained that even the larvae can infect when they ingest feces from infected imagines or feed on their dead bodies. Transovarial transmission is suspected but not proved.

The disease occurs in Southern Europe (South Russia, the Balkans, Italy, South France), North Africa, Central Asia, and India, but not on the American continent (despite the presence of blood-sucking phlebotomi), with two annual peaks (June and September).

Prophylactic measures are effective, especially if modern insecticides are systematically sprayed in houses and to a distance of 100-200 m around them. Above all, possible hatching places (garbage, compost piles, dung, etc.) should be removed or sanitized.

10. Newly Found and Suspected Arboviruses in Europe

(a) Čalovo Virus

In 1960 Bárdos and Čupková isolated in Slovakia (from a pool of Anopheles maculipennis), which they named Čalovo virus after the place of isolation. Independently, the same virus was isolated from Anopheles barbirostris as Chittoor virus in India and from Culex gelidus as Batai virus in Malaya in 1955 by Elisboy and Buescher. This virus was classified with the Bunyamwera group on the basis of serological studies. No relationship has as yet been demonstrated between Čalovo virus and human disease, but antibodies against the virus have been found from time to time in human serum [88].

(b) Kemerovo Virus

Hitherto unknown virus strains, apparently very closely related, if not identical, in antigen structure, were isolated from Ixodes persulcatus in West Siberia [38a] and from Ixodes ricinus in Slovakia [96c, 64a] and Finland [25a]. They are called Kemorovo viruses after the place where they were first discovered.

Kemerovo virus is not related antigenically to the TBE complex or to other arboviruses and is therefore regarded as a still ungrouped arbovirus. This virus is less stable than the TBE virus and is pathogenic only for 1-3-day old suckling mice [106a], although it has also been primarily isolated in chick embryonal cell cultures [96d]. The virus seems to be less widespread in the focus investigated in West Siberia than the TBE virus since neutralizing antibodies in wild and domestic animals are not found as frequently as against the TBE virus. On the other hand, the tick infection rate in Slovakia is five times higher with Kemerovo virus (1.0-1.3%) than with TBE virus (0.2%) [96b].

The importance of this virus for man has not yet been clearly established. To be sure, the virus has been occasionally isolated from the fluids of patients with febrile infections and mild meningism, but there are still no precise clinical data on a causal relationship.

There are, however, human infections in which neutralizing antibodies can be demonstrated as an expression of the infection rate in healthy persons (2.8%), although to a much lesser extent than against TBE virus (83.8%), at least in the West Siberian focus [96b]. The cycle of the virus in nature is similar to that of the TBE virus since the same host species of ticks are infected by the two viruses and the virus can also be isolated from small mammals.

(c) Tahyna Virus

In 1956 in Slovakia Bardos and Danielova were the first to isolate the Tahyna virus, named after the place, from Aedes cassius and Aedes vexans [15]. This virus, identified as an arbovirus [9, 14], has been placed in the California encephalitis complex on the basis of serological evidence [351]. Subsequently, Likar in Slovenia succeeded in isolating two strains (TROICA) in the course of a survey of 5000 serum samples which behaved in the CFR like Tahyna virus [96a].

The virus appears in various parts [8] of Central and South Europe [11] and South France since antibodies can always be demonstrated in blood samples taken from bitten persons. The Danube is a preferred region. Here Aspock isolated the virus from mosquitoes. Antibodies are found in up to 60% of the population [17, 68].

Aedes vexans is the main vector. It can also be experimentally infected with Tahyna virus, with an incubation period of at least 7 days. It is safe to assume, however, that other mosquito species play an important role in the cycle of the virus because the virus cannot be carried over the winter in Aedes vexans or be transmitted in the spring by viremic heterothermic vertebrates to this first mosquito species to appear in June [4a].

The role of animal hosts as virus reservoir has not yet been fully elucidated. Birds do not seem to be part of the virus cycle [10, 169] nor is itlikely that large domestic animals or Muridae have anything to do with the spread of the virus in nature [16, 170]. However, hares and rabbits may well function as virus reservoir since hares have been found to possess a rather high degree of natural infection and a viremia has been observed in both species of animals after experimental infection [170a].

The question of the extent to which this virus is a pathogenic agent for mancannot be answered with certainty as yet. There have, to be sure, been individual cases with atypical pneumonia causally related on serological grounds to Tahyna virus [9a], but one cannot extrapolate from this that human respiratory diseases are to be expected. This must be confirmed by further research involving virus isolations.

(d) Other Suspected Viruses

Isolation of the Čalovo and Tahyna viruses was the first proof that mosquito-borne arboviruses exist in Central Europe. Other arboviruses appear to be widespread in Central Europe and, though not yet isolated, antibodies against them have been found in human sera. In this connection, the findings on antibodies against WEE and EEE virus [6, 7, 12, 94] reported in 1954 can be interpreted as evidence not for the existence of these two viruses but for a virus of Group A. This is confirmed by similar results in Jugoslavia [123], Italy [161],

and Austria [68]. Moreover, human sera from Austria were found to contain hemagglutination-inhibiting antibodies against phlebotomus fever virus (Neapolitan type) and group B viruses (INE, Ntaya, WN); they were also detected in persons who had never left the local area in which they were born. Since the latter find can be regarded on the basis of acetone treatment of the sera as absolutely specific in the sense of a positive antibody reaction, the possibility that other arboviruses will be found in Central Europe is very likely. In contrast, reliable serological evidence of suspected arboviruses does not as yet provide any indication for the existence of hemorrhagic fever viruses. There are, of course, some clinical observations on such cases in Northern Scandinavia, Hungary, and Southeast Europe, but it is by no means certain whether a uniform nosological entity sui generis is involved or these diseases are caused by arboviruses.

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